Structure–Activity Relationships in Thymidine Phosphorylase Inhibitors.[1] A Correlation Using Substituent Constants and Regression Analysis

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The structure–activity relationship of Baker's study of several types of thymidine phosphorylase inhibitors has been made using substituent constants and regression analysis. The quantitative relationships formulated support Baker's qualitative conclusions. Activity values of 6-anilinouracils calculated from a regression equation based on 19 derivatives yield values in rather good agreement with those estimated by Baker. This substantiates the premise that additivity principles can be used in the design of enzymic inhibitors.

Attempts at relating chemical structure to enzymatic binding and reaction rates in quantitative terms have frequently proved fruitless when using a single-parameter approach. Recently, several meaningful relationships have evolved[2,3] utilizing a multiparameter approach in conjunction with computerized regression analysis. Three of the more useful parameters for such studies are Hammett's σ constant[4] and analogous parameters[5] for electronic effects of substituents, Taft's E_s[6] parameter and its modifications[3,7] for steric effects of substituents, and π[8] which is related to the lipophilic character of a substituent. Thus it has been possible in many cases to delineate the relative importance of a certain parameter with regard to a specific type of biological action and relate this result to structure–activity correlations. The potential significance of such studies with regard to cancer chemotherapy along with successes in correlating the binding of molecules to enzymes with the physicochemical parameters for their substituents has prompted an examination of the factors involved in Baker's studies of the binding of pyrmidines to thymidine phosphorylase using regression analysis.

Thymidine phosphorylase (EC 2.4.2.4.) catalyzes the reversible phosphorolytic cleavage of thymidine and other pyrimidine deoxynucleosides. Thymidine phosphorylase activity has been demonstrated in a variety of sources including mouse tissue,[9] horse liver,[10] rat liver,[11] human spleen,[11] *Escherichia coli*,[12] Ehrlich ascites tumor,[13] and others.[14] Although the enzymatic reaction is reversible, the major function in human tissues appears to be catabolic and thus it has been shown to be responsible for the rapid cleavage of the potent antimetabolite,[15,16] 5-fluoro-2′-deoxyuridine to 5-fluorouracil, leading to a lowered therapeutic effectiveness of the former compound and a much greater toxicity due to the latter compound. Inhibition of this reaction should therefore increase the efficiency of this and other nucleoside antimetabolites. Since thymidine phosphorylase appears to bind specifically to the deoxyribosyl portion[17,18] of the nucleoside, and in view of the product inhibition seen with thymine, recent studies have concentrated on the modification of the pyrimidine moiety.[19–28] The extensive investigations conducted by Baker and his coworkers[20–28] have provided the data for this analysis.

Method

The inhibitory activities of the pyrimidine molecules have been reported in terms of the ratio of inhibitor to substrate affording 50% inhibition of the enzyme $[I/S]_{0.5}$. The substrate in all cases studied by Baker was 5-fluoro-2′-deoxyuridine. The relationship between this ratio and the Michaelis–Menten constant is represented by eq 1 where K_i and K_m are the enzyme–

$$\frac{K_i}{K_m} = \frac{I}{S} = \left[\frac{I}{S}\right]_{0.5} \quad (1)$$

inhibitor dissociation constant and the enzyme–substrate dissociation constant, respectively. I is the concentration of inhibitor and S is the concentration of substrate. It has been shown that $[I/S]_{0.5}$ for two inhibitors is related to the differences in free energy of the two molecules.[29] As a first approximation, the free energy ($\Delta G_{BR}°$) in a standard biological response can be factored as follows:

(1) This work was supported by Grant CA 11110 from the National Institutes of Health.

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(22) B. R. Baker and M. Kawazu, *ibid.*, **10**, 311 (1967).
(23) B. R. Baker and M. Kawazu, *ibid.*, **10**, 313 (1967).
(24) B. R. Baker and M. Kawazu, *ibid.*, **10**, 316 (1967).
(25) B. R. Baker, M. Kawazu, and J. D. McClure, *J. Pharm. Sci.*, **56**, 1081 (1967).
(26) B. R. Baker and M. Kawazu, *ibid.*, **56**, 1086 (1967).
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(28) B. R. Baker and W. Rzeszotarski, *ibid.*, **11**, 639 (1968).
(29) B. R. Baker, "Design of Active-Site-Directed Irreversible Enzyme Inhibitors," Wiley, New York, N. Y., 1967, pp 202–204.

$$\Delta G_{BR}^{\circ} = \Delta G_{hydrophobic}^{\circ} + \Delta G_{electronic}^{\circ} + \Delta G_{steric}^{\circ} \sim \ln k_{BR} \quad (2)$$

In eq 2 k_{BR} is a rate or equilibrium constant for the rate-limiting chemical or physical reaction which ultimately causes an observed biological response. The effect of substituents on the free-energy change of a reference molecule can be represented as in eq 3. One

$$\delta_X \Delta G_{BR}^{\circ} = \delta_X \Delta G_{hydrophobic}^{\circ} + \delta_X \Delta G_{electronic}^{\circ} + \delta_X \Delta G_{steric}^{\circ} \sim \delta_X \log k_{BR} \quad (3)$$

can use constants from model systems in a Hammett-type approach to evaluate the effect of substituents on k_{BR} as in eq 4. In eq 4, C_X is the molar concentration of compound with substituent X producing an equivalent biological response such as $[I/S]_{0.5}$ under the assay conditions used in the experiment. The constants k, ρ, k', and k'' are fixed for a given system and are evaluated by the method of least squares. π values for the substituents were estimated from previously determined values for the functional groups while that portion of the molecule which was held constant was assumed to make a constant contribution to the overall partition coefficient and was neglected. For example, the change in hydrophobic binding for the series of 1-substituted uracils was assumed to be adequately accounted for by the π constants for the 1 substituents alone. The Hammett σ values or modified σ values were taken from the sources indicated. Recently, Swain and Lupton[30] have introduced two new electronic parameters, $\mathcal{R}$ and $\mathcal{F}$. $\mathcal{R}$, the resonance constant, and $\mathcal{F}$, the field constant, are used for correlating substituent effects regardless of substituent position. Another electronic parameter receiving more attention is polarizability, P_E, which has been used in some recent correlations.[31,32] The steric parameters used were taken from Taft's E_s values or from E_s values of certain substituents based on van der Waal's radii.

$$\log k_{BR} \equiv \log \frac{1}{C_X} = k\pi_X + \rho\sigma_X + k'E_{s_X} + k'' \quad (4)$$

In order to assess the additive nature of π for vicinal Cl groups as well as the π value for Cl in the *ortho* position, partition coefficients for several aniline derivatives were measured. In each case the standard derivative is from 4 determinations made at different concentrations. Log P values found for the octanol-H_2O systems are as follows: 2-chloroaniline, 1.90 ± 0.01; 2,3-dichloroaniline, 2.78 ± 0.02; 3,4-dichloroaniline, 2.69 ± 0.01. The rather constant character of π_{Cl} can be seen from the following calculations:

$$\pi_{2\text{-Cl}} = \log P_{\text{2-chloroaniline}} - \log P_{\text{aniline}} = 1.90 - 0.90 = 1.00$$

$$\pi_{2\text{-Cl}} = \log P_{\text{2,3-dichloroaniline}} - \log P_{\text{3-chloroaniline}} = 2.78 - 1.88 = 0.90$$

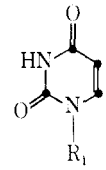
$$\pi_{3\text{-Cl}} = \log P_{\text{3-chloroaniline}} - \log P_{\text{aniline}} = 1.88 - 0.90 = 0.98$$

$$\pi_{3\text{-Cl}} = \log P_{\text{2,3-dichloroaniline}} - \log P_{\text{2-chloroaniline}} = 2.78 - 1.90 = 0.88$$

The hydrophobic character of Cl changes relatively little, even when bracketed by an NH_2 and Cl function in 2,3-dichloroaniline.

Tables I–V contain data on substituent changes at the 1, 5, and 6 positions of the uracil ring. In each case a number of derivatives were omitted from the correlation due to lack of suitable substituent constants or because the predicted activity grossly deviated from observed results even when suitable substituent constants were available.

TABLE I
INHIBITORS OF THYMIDINE PHOSPHORYLASE
1-SUBSTITUTED URACILS



R_1[a]	P_E[b]	π	σ^*[c]	Log 1/C Obsd[d]	Log 1/C Calcd[e]	Δ Log 1/C
$(CH_2)_5C_6H_5$	50.53	4.03	−0.04[f]	−0.32	−0.34	0.02
$(CH_2)_4C_6H_5$	45.87	3.53	−0.04[f]	−0.60	−0.61	0.01
$CH_2C_6H_5$	31.89	2.63	0.22	−0.76	−1.09	0.33
$CH_2CH_2C_6H_5$	36.55	3.13	0.08	−0.80	−0.82	0.02
$(CH_2)_3C_6H_5$	41.21	3.03	0.02	−1.11	−0.87	0.24
n-C_5H_{11}	25.25	2.50	−0.14[f]	−1.15	−1.16	0.01
i-C_6H_{14}	29.91	2.80	−0.06[f]	−1.17	−1.00	0.17
Cyclo-C_5H_9	23.19	2.14	−0.20	−1.28	−1.35	0.07
i-C_5H_{11}	25.25	2.30	−0.06[f]	−1.30	−1.26	0.04
n-C_4H_9	20.59	2.00	−0.13	−1.35	−1.42	0.07
CH_3	6.57	0.50	0.00	−2.30	−2.23	0.07

[a] Omitted $CH_2C_6H_4CONH_2$-*p*, $CH_2C_6H_4NHCOCH_3$-*p*, $(CH_2)_4$-OH, CH_2CH_2OH. [b] From A. I. Vogel, W. T. Cresswell, G. H. Jeffrey, and J. Leicester, *J. Chem. Soc.*, 514 (1952). [c] From ref 5, p 222. [d] Estimated values. [e] From ref 18 and 20. [f] Calculated using eq 6.

Results and Discussion

1-Substituted Uracils.—Analysis of Table I on 1-substituted uracils has resulted in eq 5–7. The figures in parentheses are the 95% confidence intervals, n is the number of data points, r is the correlation coefficient, and s is the standard deviation. Equation 5, with a term for polarizability, accounts for a large part of the variance; however, eq 6, with a term in

	n	r	s	
$\log \frac{1}{C} = 0.038(\pm 0.01)P_E - 2.261(\pm 0.40)$	11	0.921	0.212	(5)
$\log \frac{1}{C} = 0.535(\pm 0.12)\pi - 2.495(\pm 0.33)$	11	0.959	0.153	(6)
$\log \frac{1}{C} = 1.052(\pm 3.30)\sigma^* - 1.070(\pm 0.37)$	11	0.234	0.527	(7)

(30) C. G. Swain and E. C. Lupton, Jr., *J. Amer. Chem. Soc.*, **90**, 4328 (1968).
(31) D. Agin, L. Hersh, and D. Holtzman, *Proc. Nat. Acad. Sci. U. S.*, **53**, 952 (1965).
(32) C. Hansch and E. Coats, *J. Pharm. Sci.*, **59**, in press (1970).

π, has a lower standard deviation and higher correlation than eq 5. Equation 7, with the electronic parameter σ^*, shows that electronic factors alone are not important for effective binding by the 1 substituents. Combinations of π, P_E, and σ^* did not result in significant improvements over eq 6 as demonstrated by the F test ($\alpha \leq 0.10$). The π values for the phenylpropyl, phenylbutyl, and phenylpentyl derivatives were adjusted to account for folding[3,33] that can occur with these compounds. The implication here is that when the connecting alkyl chain between the uracil ring and the Ph ring reaches a certain length, the two π systems are allowed to interact, thus lowering their π values. If this adjustment were not considered and "normal" π values were used, eq 8, of considerably

$$\log \frac{1}{C} = 0.421(\pm 0.14)\pi - 2.266(\pm 0.40) \quad n = 11 \quad r = 0.920 \quad s = 0.213 \quad (8)$$

poorer correlation, would result.

The need for an anion at N_1 is mentioned in the section on 5-substituted uracils. When the H-1 is replaced by small alkyl or aralkyl groups, binding ability is lost due to the loss of the anion at this position. Binding and, hence, inhibition can be regained by bridging this polar area with alkyl or aralkyl groups sufficiently long enough to reach a hydrophobic area. Four compounds were omitted from the correlation. The 2-hydroxyethyl and the 5-hydroxypentyl deviated markedly from their observed activities even though suitable substituent constants were available. The other two, $CH_2C_6H_4CONH_2$-*p* and $CH_2C_6H_4NHAc$-*p*, were omitted because of the lack of suitable substituent constants. Of the 11 compounds correlated, π accounts for most of the variance and indicates that binding in this area depends on the hydrophobic character of the substituent. Nevertheless, polarizability should be kept in mind when considering substituents at the 1 position.

5-Substituted Uracils.—Table II contains the substituent constants for 5-substituted uracils including pK_a for the N_1 H. Equations 9–11 show that π alone

$$\log \frac{1}{C} = 0.219(\pm 0.50)\pi - 0.279(\pm 0.46) \quad n = 8 \quad r = 0.401 \quad s = 0.494 \quad (9)$$

$$\log \frac{1}{C} = 1.40(\pm 0.92)\sigma_I - 0.548(\pm 0.34) \quad n = 8 \quad r = 0.834 \quad s = 0.297 \quad (10)$$

$$\log \frac{1}{C} = -0.273(\pm 0.15)pK_a + 2.178(\pm 1.31) \quad n = 8 \quad r = 0.879 \quad s = 0.257 \quad (11)$$

is not very significant, but as Baker[23] has pointed out, the degree of ionization at N_1 is quite important. It is interesting to note that σ_I gives almost as good a correlation as pK_a. This illustrates the versatility of σ constants which in this instance gave good results in a complex heterocyclic system far removed structurally from that system in which they were derived.

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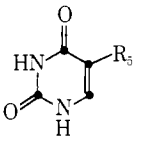
Table II
Inhibitors of Thymidine Phosphorylase
5-Substituted Uracils



R_5	π[b]	pK_a[c]	σ_I[e]	−Log 1/C− Obsd[f]	−Log 1/C− Calcd[g]	Δ Log 1/C
NO_2	0.33	5.3	0.76	0.66	0.72	0.06
Br	0.89	8.0	0.45	0.35	0.11	0.24
F	0.25	8.0	0.52	−0.11	−0.02	0.09
CH_3	0.50	9.9	−0.08	−0.28	−0.48	0.20
C_6H_5	2.13	9.9[d]	0.08	−0.30	−0.15	0.15
$COCH_3$	−0.55	9.3[d]	0.18	−0.52	−0.54	0.02
H	0.00	9.5	0.00	−0.59	−0.48	0.11
NH_2	−0.84	10.0[d]	0.05	−0.85	−0.79	0.06

[a] Omitted N_2^+ and CO_2H. [b] From *o*-phenols where possible. [c] pK_a for N_1 H, enzyme assay at pH 5.9; see ref 23. [d] Estimated pK_a. [e] From R. W. Taft, Jr., Elton Price, Irwin R. Fox, Irwin C. Lewis, K. K. Andersen, and George T. Davis, *J. Amer. Chem. Soc.*, **85**, 709 (1963). [f] From ref 22 and 23. [g] Calculated using eq 13.

Addition of a term in π to eq 10 and 11 yields eq 12 and 13. An F test indicates that eq 12 is not a significant improvement over eq 11 ($F_{1,5} = 8.18$). The negative coefficient associated with pK_a means that the lower the pK_a, the better the inhibitor. The coefficients in eq 13 suggest that an ideal group would possess strong electron withdrawal while being lipophilic enough to take advantage of the positive coefficient associated with π in eq 13. Such a group is the SO_2CF_3 group proposed by Baker.[23] The Hammett σ for SO_2CF_3 is 0.93 compared with NO_2 which is 0.78, while π for SO_2CF_3 is 0.93 (from phenoxyacetic acid system) compared with NO_2 which is 0.33 (from *o*-nitrophenol system). By assuming 90% ionization for 5-trifluoromethylsulfonyluracil (based on pH 5.9 for the enzyme assay[23]), the predicted activity calculated from eq 13 would be log $1/C = 0.95$ or $[I/S]_{0.5} = 0.11$ which compares to $[I/S]_{0.5} = 0.22$ for the NO_2 group. The dependence on the pK_a term in eq 13 would seem to indicate that an anion at N_1 is important for binding. Since 1-methyluracil shows a 50-fold loss in binding[23] compared with uracil, an anion at N_1 appears to be important for binding.

$$\log \frac{1}{C} = 1.368(\pm 0.82)\sigma_I + 0.194(\pm 0.27)\pi - 0.606(\pm 0.31) \quad n = 8 \quad r = 0.906 \quad s = 0.249 \quad (12)$$

$$\log \frac{1}{C} = -0.269(\pm 0.11)pK_a + 0.206(\pm 0.18)\pi + 2.080(\pm 0.93) \quad n = 8 \quad r = 0.956 \quad s = 0.174 \quad (13)$$

6-Substituted Benzyluracils.—Table III contains several benzyluracils along with the substituent constants used for correlating their biological activity. The terms E_s^m and E_s^p refer to Taft's steric parameter[6] or a modification of the steric parameter[3,7] for the *meta* and *para* positions on the benzyl moiety, respectively. Equation 14, with a term for E_s^m and

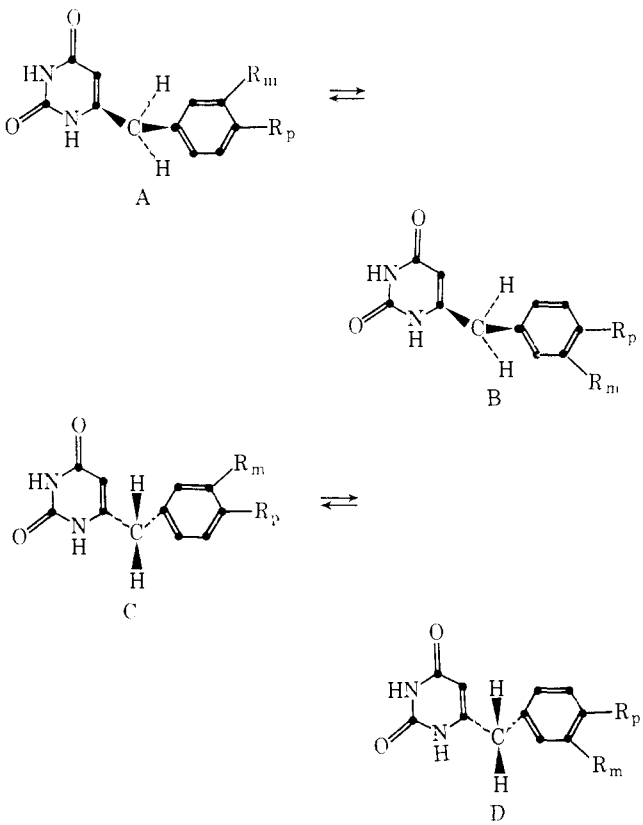
$$\log \frac{1}{C} = -0.585(\pm 0.19)E_s^m - 0.349(\pm 0.27)E_s^p + 1.721(\pm 0.28) \quad (14)$$

$n = 7$ $r = 0.973$ $s = 0.193$

$$\log \frac{1}{C} = 0.711(\pm 2.12)\pi_m + 0.409(\pm 1.51)\pi_p + 1.236(\pm 0.80) \quad (15)$$

$n = 7$ $r = 0.523$ $s = 0.717$

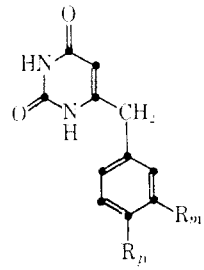
E_s^p, accounts for nearly all of the variance ($r = 0.973$), while the addition of terms in π or σ do not significantly improve the correlations. The negative sign associated with the steric parameters in eq 14 suggests that inhibiting ability is enhanced by increasing steric bulk. The difference in the coefficients for the *meta* and *para* steric parameters is indicative that steric bulk plays a slightly larger role in increasing the binding at the *meta* position than it does at the *para* position. There are two conformations in which these compounds can exist. The first conformation would place the pyrimidine ring and phenyl ring in a V with the methylene bridge at the apex while the two rings are roughly "parallel." The second conformation would place one of the rings perpendicular to the other. Various rotomers of these conformations such as the following are possible:



From an inspection of Fisher–Hirschfelder–Taylor space filling models, the conformations B and D were ruled unlikely because the *meta* substituents project into the vicinity of the hydrophobic area around the N_1 position. The conformations A and C are indistinguishable by this analysis; however, both have the bulky *meta* substituents far away from the hydrophobic area around the N_1 position (established by eq 6). Four rotomers can exist in the case where one ring is perpendicular to the other ring. From the series of benzyl derivatives studied, it is not possible to draw any firm conclusions as to which of these rotomers are important in binding to the enzyme. The use of regression analysis with rigid analogs would be necessary to shed light on this problem. Seven molecules were omitted form the correlation, six of them due to the lack of suitable substituent constants. The seventh compound, 6-(3-nitro-4-fluoro)benzyluracil, for which substituent constants were available, deviated markedly from the other compounds studied.

Table III

Inhibitors of Thymidine Phosphorylase 6-Substituted Benzyluracils



R_m[a]	R_p	E_s^m[b]	E_s^p[c]	Lng 1/C Obsd[d]	Lng 1/C Calcd[e]	|Δ Lng 1/C|
NO_2	NH_2	−1.28	0.68	2.20	2.23	0.03
NO_2	H	−1.28	1.24	2.07	2.04	0.03
H	NO_2	1.24	−1.28	1.48	1.44	0.04
H	CH_3	1.24	0.00	1.02	1.00	0.02
H	F	1.24	0.78	0.89	0.72	0.17
H	H	1.24	1.24	0.66	0.56	0.10
H	NH_2	1.24	0.68	0.43	0.76	0.33

[a] Omitted $R_m = NO_2$, $R_p = F$; $R_m = H$, $R_p = SO_2Cl$; $R_m = H$, $R_p = SO_2NH_2$; $R_m = H$, $R_p = HNCOCH_2Br$; $R_m = HNCOCH_2Br$, $R_p = H$; $R_m = NO_2$, $R_p = HNAc$; $R_m = H$, $R_p = HNAc$. [b] E_s value for *meta* substituent. [c] E_s value for *para* substituent. [d] From ref 22, 24, and 26. [e] Calculated using eq 14.

6-Substituted Uracils.—The compounds listed in Table IV were correlated by eq 22. Various combina-

$$\log \frac{1}{C} = 0.014(\pm 0.27)\pi - 0.068(\pm 0.52) \quad (16)$$

$n = 11$ $r = 0.039$ $s = 0.591$

$$\log \frac{1}{C} = 0.016(\pm 0.03)P_E - 0.328(\pm 0.62) \quad (17)$$

$n = 11$ $r = 0.391$ $s = 0.544$

$$\log \frac{1}{C} = -0.955(\pm 1.20)\mathfrak{R} - 0.197(\pm 0.39) \quad (18)$$

$n = 11$ $r = 0.515$ $s = 0.507$

$$\log \frac{1}{C} = 0.896(\pm 0.91)\mathfrak{F} - 0.225(\pm 0.37) \quad (19)$$

$n = 11$ $r = 0.597$ $s = 0.474$

$$\log \frac{1}{C} = 0.961(\pm 0.70)\mathfrak{F} - 1.046(\pm 0.87)\mathfrak{R} - 0.397(\pm 0.32) \quad (20)$$

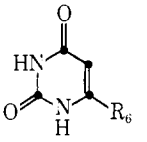
$n = 11$ $r = 0.820$ $s = 0.359$

$$\log \frac{1}{C} = 0.139(\pm 0.16)\pi - 1.133(\pm 0.76)\mathfrak{R} + 1.177(\pm 0.65)\mathfrak{F} - 0.620(\pm 0.37) \quad (21)$$

$n = 11$ $r = 0.893$ $s = 0.302$

TABLE IV

INHIBITORS OF THYMIDINE PHOSPHORYLASE 6-SUBSTITUTED URACILS

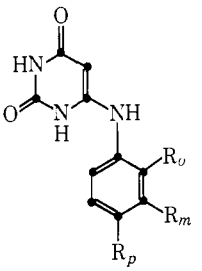


R_6[a]	P_E	$\mathcal{R}$[b]	$\mathcal{F}$[b]	Log 1/C Obsd[d]	Log 1/C Calcd[e]	|Δ Log 1/C|
OC_6H_5	27.32	−0.74	0.75	1.23	1.28	0.05
CH_3SO_2	9.21	0.22	0.90	0.28	0.22	0.06
$CH_2CH_2C_6H_5$	34.98	−0.11[c]	−0.06[c]	0.22	−0.04	0.26
NH_2	3.52	−0.68	−0.04	0.17	0.02	0.15
$(CH_2)_3C_6H_5$	39.64	−0.11[c]	−0.07[c]	−0.04	0.04	0.08
n-C_5H_{11}	23.68	−0.11[c]	−0.07[c]	−0.04	−0.26	0.22
CF_3	4.32	0.19	0.63	−0.08	−0.14	0.06
n-C_3H_7	14.36	−0.11	−0.07	−0.40	−0.43	0.03
C_6H_5	25.36	−0.09	0.14	−0.40	−0.02	0.38
H	1.68	0.00	0.00	−0.59	−0.69	0.10
CH_3	5.00	−0.14	−0.05	−0.91	−0.55	0.36

[a] Omitted NHPh, SPh, SO_2Ph, COPh, CHOHPh, $NHCH_2Ph$, $N(CH_3)C_6H_5$, $NHCH_2CH_2Ph$. [b] From ref 30. [c] Calculated values from ref 30. [d] From ref 22, 26, and 27. [e] Calculated using eq 22.

TABLE V

INHIBITORS OF THYMIDINE PHOSPHORYLASE 6-SUBSTITUTED ANILINOURACILS



R_o[a]	R_m	R_p	E_s[b]	π_o	π_m	π_p	Log 1/C Obsd[c]	Log 1/C Calcd[d]	|Δ Log 1/C|
Cl	Cl	H	0.27	0.94	0.94	0.00	3.04	2.99	0.05
CH_3	CH_3	H	0.00	0.68	0.50	0.00	2.65	2.35	0.30
H	C_6H_5	H	1.24	0.00	2.13	0.00	2.63	2.71	0.08
CH_3	H	CH_3	0.00	0.68	0.00	0.50	2.43	2.20	0.23
H	H	C_6H_5	1.24	0.00	0.00	2.13	2.32	2.07	0.25
Cl	H	H	0.27	1.00	0.00	0.00	2.28	2.38	0.10
CH_3	H	H	0.00	0.68	0.00	0.00	2.11	1.98	0.13
C_2H_5	H	H	−0.07	1.00	0.00	0.00	2.11	2.38	0.27
H	Cl	H	1.24	0.00	0.98	0.00	1.81	1.86	0.05
H	H	OC_2H_5	1.24	0.00	0.00	0.50	1.78	1.36	0.42
H	H	n-C_4H_9	1.24	0.00	0.00	2.00	1.73	2.01	0.28
H	H	Br	1.24	0.00	0.00	1.02	1.60	1.59	0.01
H	CH_3	H	1.24	0.00	0.50	0.00	1.56	1.51	0.05
H	H	Cl	1.24	0.00	0.00	0.98	1.46	1.57	0.11
H	H	C_2H_5	1.24	0.00	0.00	1.00	1.43	1.58	0.15
OCH_3	H	H	0.69	−0.02	0.00	0.00	1.30	1.12	0.18
H	H	CH_3	1.24	0.00	0.00	0.50	1.28	1.36	0.08
OC_2H_5	H	H	0.69	0.35	0.00	0.00	1.23	1.58	0.35
H	H	H	1.24	0.00	0.00	0.00	1.00	1.14	0.14

[a] Omitted p-C_4H_9-t, 2,6-Me_2, 2,5-Me_2, 2,3-benzo, 3,4-benzo, 4,5(2,3-naphtho), N^6Me, cyclohexylamino. [b] E_s values for *ortho* substituents. See ref 3. [c] From ref 28. [d] Calculated using eq 23.

$$\log \frac{1}{C} = 0.018(\pm 0.01)P_E - \quad 11 \quad 0.928 \quad 0.259 \quad (22)$$
$$0.930(\pm 0.63)\mathcal{R} + 1.078(\pm 0.51)\mathcal{F} - 0.72(\pm 0.34)$$

tions of π, P_E, $\mathcal{R}$, and $\mathcal{F}$ involving two parameters resulted in eq 16–21. An F test ($F_{2,7} = 4.77$) demonstrates that eq 22 is a significant improvement over eq 20. The large positive sign associated with the field constant, $\mathcal{F}$, in eq 22 suggests that binding is increased by electron withdrawal from N_1, a finding similar to that for the 5 substituents. Polarizability seems to play a more important part in the binding than does π. The large negative coefficient for the resonance constant, $\mathcal{R}$, suggests that electron donors on the inhibitor may be important to some electron acceptor(s) on the enzyme. Eight compounds were omitted from the correlation due to lack of suitable substituent constants.

6-Substituted Anilinouracils.—Table V contains 19

anilines substituted on the 6 position of the uracil ring. Equations 23-25 correlate the activity of the 6-anilinouracils, with eq 23 giving the best fit. Equation 25,

		n	r	s	
$\log \frac{1}{C} =$	$1.234(\pm 0.32)\pi_m +$	19	0.929	0.229	(23)
	$0.734(\pm 0.23)\pi_m + 0.435(\pm 0.20)\pi_p +$				
	$1.144(\pm 0.22)$				
$\log \frac{1}{C} =$	$1.310(\pm 0.37)\pi_o +$	19	0.893	0.270	(24)
	$0.558(\pm 0.21)\pi_{m+p} + 1.114(\pm 0.25)$				
$\log \frac{1}{C} =$	$-0.993(\pm 0.31)E_s^o +$	19	0.878	0.287	(25)
	$0.630(\pm 0.23)\pi_{m+p} + 2.238(\pm 0.26)$				

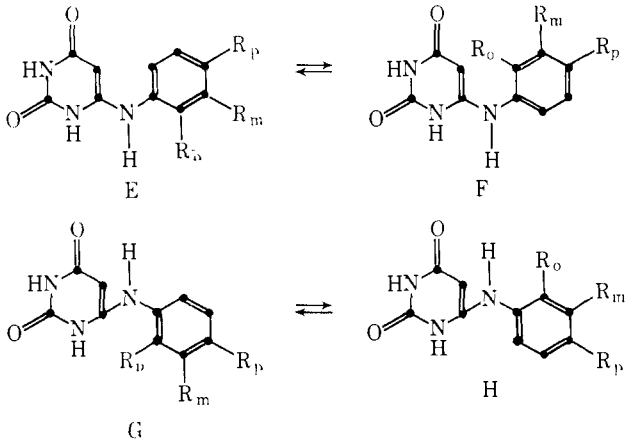
with the E_s^o term replacing the π_o term of eq 24, gave essentially the same correlation. It is therefore not possible to say with any certainty that only hydrophobic effects are involved.[34] A steric component may also be present. The positive signs in eq 23 for the hydrophobic parameters indicate that increasing the lipophilic character of a substituent increases binding in the order $\pi_o > \pi_m > \pi_p$. No significant improvement in eq 23 was obtained by the addition of various electronic parameters.

In order to test for the steric component, eq 26

		n	r	s	
$\log \frac{1}{C} =$	$-0.445(\pm 0.54)E_s^o +$	19	0.943	0.214	(26)
	$0.712(\pm 0.70)\pi_o +$				
	$0.785(\pm 0.22)\pi_m +$				
	$0.482(\pm 0.20)\pi_p +$				
	$1.618(\pm 0.61)$				

was devised which includes the E_s^o term with terms for π_o, π_m, and π_p. The coefficients for π_o and π_m are essentially the same and suggest that groupings at these positions bind to the same hydrophobic site. The difference in the coefficients for π_o in eq 23 and 26 may be the result of intramolecular interactions of the *ortho* substituent represented by the E_s^o term.

Conformations that the 6-anilinouracils can assume are:



(34) The squared correlation coefficient for the correlation between E_s^o and π_o for the set of substituents considered in eq 24 and 25 is 0.849. Such high interrelation between steric and hydrophobic character makes it difficult to delineate the substituent effect in this position. Care should be taken in the design of derivatives so that good spread in π, E_s, and σ is obtained for substituents at each position.

Since an anion at N_1 seems to be important, then conformations E and G might be ruled out because the hydrophobic *ortho* substituent closely approaches the binding point for the N_1 anion. Four other conformations can exist in which the phenyl ring of the anilino moiety is perpendicular to the pyrimidine ring. In two of these conformations the hydrophobic *ortho* substituent will be crowded close to the pyrimidine ring and hence may hydrophobically interfere with binding of the N_1 anion, and therefore these conformations may be ruled out. The other two conformations would place the hydrophobic *ortho* substituent away from the pyrimidine ring. It is not possible from this analysis to distinguish between these last two conformations or conformations represented by F and H. Eight compounds were omitted from the correlation, 7 because of the lack of suitable substituent constants, while the *p*-*t*-Bu was omitted because it deviated badly from all the correlations studied. The addition of an E_s^p term did not improve the correlation, and some other explanation is needed to account for the large deviation of the *p*-*t*-Bu derivatives.

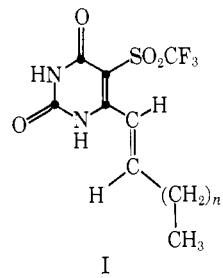
Baker,[35] assuming an additivity of substituent effects, predicted that 6-(2,3-dichloro-4-ethoxy)anilinouracil and 6-(2,3-dichloro-4-phenyl)anilinouracil would have log $1/C$ values of 3.82 and 4.35, respectively. The values of 3.21 and 3.92 calculated for these compounds using eq 23 are in rather good agreement with Baker's estimates.

From all the sets of inhibitors studied, some compounds were omitted because they did not fit the correlations. These compounds were not omitted for the sake of improving the correlation at the expense of data points, but because these compounds deviated badly from other members of a series even when reliable substituent constants were available. This suggests that these compounds may be acting by a different mechanism or producing some change not caused by the other members of a series. For example, 6-(3-nitro-4-fluoro)benzyluracil has log $1/C = 1.06$, yet this compound deviated the most from all the correlations studied for the benzyluracils. Assuming that the site of binding is the same as for the other benzyluracils, then one explanation for the erratic behavior might be a nucleophilic attack on 6-(3-nitro-4-fluoro)benzyluracil producing a new compound that binds differently or somehow inactivates the enzyme differently.

A systematic study of 1,5-disubstituted uracils was attempted, but no meaningful correlations resulted due to the small number of data points and the lack of suitable substituent constants available for some of the substituents.

More data points were available for a study of 5,6-disubstituted uracils, but again no meaningful correlations were obtained by using π, P_E, or σ_I in various combinations. The lack of pK_a data for the 5,6-disubstituted uracils precludes a complete study of these compounds. Nevertheless, the findings from 5- and 6-monosubstituted uracils suggest that effective 5,6-disubstituted uracils would possess electron-withdrawing groups at the 5 position and 6 substituents capable of acting by a combination of electron donor through resonance and electron withdrawal through induction. In designing possible candidate inhibitors based on this study, advantage should be taken from the finding

that a hydrophobic area exists beyond the N_1 position and at the 5 position, and that compounds with an acidic hydrogen at the 1 position make good inhibitors. Since the 6-anilinouracils show good inhibition, incorporation of this moiety would be advantageous. The following compounds would be predicted to be good inhibitors of thymidine phosphorylase. The long alkyl chain in each compound would be expected to bridge to the hydrophobic area beyond the N_1 position. The 5-SO_2CF_3 group would provide hydrophobic character as well as lowering the pK_a of the N_1 hydrogen. The dichlorophenyl moiety would provide binding in the area where the 6-anilinouracils bind. This study does



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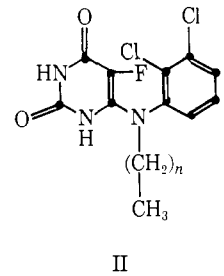

II

support in a quantitative way the qualitative findings of Baker and coworkers. It also offers ideas for the development of more effective inhibitors.

Mixed Bifunctionality. III. Antitumor Activity of Sesame Oil Solutions of Simple Alkylating Derivatives of Polynuclear Hydrocarbons[1]

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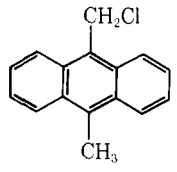
Antitumor activity of chloromethyl aromatic hydrocarbons is enhanced by administration in sesame oil solution compared with saline suspension. Microgram amounts of the most active compounds are curative in the Ehrlich mouse ascites tumor. Structural variation of the polycyclic aromatic radical has been related to antitumor activity. These relationships only partially correspond with those when mustard groups rather than the chloromethyl group furnish the alkylating function. The previously noted high activity of chloromethyl aromatic hydrocarbon *vs.* the mustard-resistant S-37 tumor has been studied in detail.

We have previously reported the discovery that antitumor activity is conferred on a monofunctional N mustard,[2,3] on S half mustard,[4] and on a simpler alkylating function[5] by the presence of a polynuclear moiety in the same molecule. Several simple chloromethyl aromatic hydrocarbons were among the most potent compounds. This discovery was surprising since these are hydrophobic, insoluble chemicals which were given as fine suspensions in saline to tumor-bearing mice.

In an effort to determine whether greater *in situ* availability would affect the antitumor activity of such compounds as I–III, they were injected intraperitoneally as solutions in sesame oil into mice bearing ascites tumors.[2-5] This mode of administration in fact



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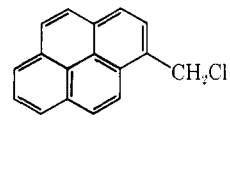

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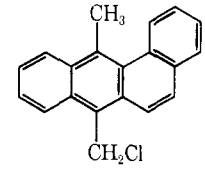

III

markedly increased both the activity and the toxicity of I–III compared with these properties when I–III were given in suspension. In view of this enhancement of potency, further structural variation of the aromatic group was studied (see Table I and section on Biological Results). In addition, the previously noted efficacy of some of these compounds against the mustard-resistant S-37 tumor[5] has been examined (see Table II).

To obtain the previously unreported compounds in Table I, direct chloromethylation was not attempted, since it had been found that the impurity from even a small amount of excess chloromethylation can give a false enhancement of activity.[5] Where possible, the aldehyde was the preferred intermediate, followed by (1) reduction either with $LiBH_4$ or $NaBH_4$, and (2) action of dry HCl. Several of the required aldehydes are known, and formylation of 2,9-dimethylanthracene gave the 10-carboxyaldehyde. However, 1,9-dimethylanthracene gave an intractable tar. The only method found to obtain this and other hydroxymethylanthracenes bearing alkyl substituents in the outer rings was *via* the ICH_2 derivatives available from the anthraquinone.[6] Reaction of these iodo compounds with moist Ag_2O gave variable yields of the $HOCH_2$ compound. Dry HCl yielded the $ClCH_2$ compound in every case except the same 1,9-dimethyl derivative. In one case, **7** in Table I, PCl_3 in C_6H_6 was employed.[7] Table III lists the intermediate $HOCH_2$ compounds not previously reported.

Experimental Section

Melting points were taken in open capillary tubes in a Hershberg apparatus using total immersion thermometers and are reported as uncorrected values. Where analyses are indicated

(1) Supported by Research Grants CA-02975, CA-06927, and FR-05539 from the National Institutes of Health, U. S. Public Health Service and by an appropriation from the Commonwealth of Pennsylvania.

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