It may be seen that dFU-5′-IIP has a greater activity on these cells than dFU-3′-IIP. Figure 1 permits a comparison of the effectiveness of the compounds on a molar equivalent instead of milligram basis as used in the calculation of ID_{50} values. Comparative ratios (treated cells: control cells) calculated from data obtained on day 3 at a drug concentration of 10^{-6} *M*, are FUDR, 0.11; dFU-3′-IIP, 0.53; dFU-5′-IIP, 0.14 or 11.0%, 53.0%, and 14% inhibition of control cell growth, respectively. Day 3 was chosen because by the 4th day there may be some loss of cells, especially in the control tubes, due to overcrowding and exhaustion of nutrients from the media. In addition, as anticipated theoretically, dFU-5′-IIP was hydrolyzed during cell growth to the purple-blue diiodoindigo which could be observed microscopically inside the cells.

Structure–Antitumor Activity Correlation of Some Schiff Bases

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Schiff bases, containing the >C=N group, are known to have slight antitumor activities;[1] more of these compounds have been synthesized in order to find compounds with greater antitumor activities. The Cancer Chemotherapy National Service Center has screened these compounds against lymphoid leukemia L1210 in the mouse and intramuscular Walker sarcoma 256 in the rat. The antitumor results are reported here with attempts to correlate these activities with the chemical structures of the compounds.

The biological activities of these compounds are difficult to determine because of their rapid hydrolysis in aqueous solution. In some cases the half-life of the uncatalyzed hydrolysis at 25° at pH 7 is only 12 min.[2] Unless the solutions for injections are prepared shortly before use they contain an equilibrium mixture of the Schiff base and its hydrolysis products.

None of the compounds listed in Table I have activity against mouse leukemia L1210. However several of them slow the growth of the intramuscular Walker sarcoma of the rat, in one case to 58% of the tumor growth of the untreated animals. Effectiveness against intramuscular Walter sarcoma of the rat is measured by weights of tumors of treated rats (T) compared with the tumors of control rats (C); the value of T/C must be 0.53 or less for significant activity.[3]

Structure–Activity Relationships.—In applying the Hansch[4] method of correlation to these data the biological activity was expressed in various ways: (1) T/C, (2) C/T, (3) dose in grams for 20% response, (4) log (C/T at the maximum tolerated dose), (5) log (dose in grams for 20% response), (6) dose in moles for 20% response, and (7) log (dose in moles for 20% response). Correlation by each method separately showed the best fit to the data with 1, 2, and 4; log (C/T at the MTD) was chosen because of the nature of Hansch's equation. If more data on the dose–activity curve were available one of the other values probably would have given better results, especially those involving dose levels for a given response. The values in Table I of σ for the *meta* and *para* positions were those compiled by Jaffe[5] and those for the *ortho* position were taken from the work of Barlin and Perrin[6] on the strengths of substituted benzoic acids. Although other values of σ were used in these correlations, these particular values gave the best results. The values of π were taken from a list published by Fujita, *et al.*,[7] based on a study of the partition between 1-octanol and H_2O of 203 mono- and disubstituted benzenes. The values of π in Table I are those for phenoxyacetic acids since these gave the best correlation with our data.

The results of linear regression analysis of each of these variables, individually and collectively, follow (r is the correlation coefficient and s is the standard deviation from regression):

	r	s
$\log C/T = -0.0068\sigma + 0.042$	0.063	0.105
$\log C/T = 0.062\sigma' + 0.0081$	0.537	0.089
$\log C/T = 0.0048\sigma + 0.063\sigma' + 0.0032$	0.539	0.091
$\log C/T = 0.082\pi + 0.045$	0.313	0.100
$\log C/T = -0.150\pi' + 0.00095$	0.531	0.089
$\log C/T = 0.026\pi - 0.138\pi' + 0.0066$	0.539	0.091
$\log C/T = 0.185\pi^2 + 0.005$	0.237	0.103
$\log C/T = 0.463\pi'^2 - 0.048$	0.576	0.086
$\log C/T = 0.239\pi^2 + 0.491\pi'^2 - 0.092$	0.651	0.082
$\log C/T = 0.0062\sigma + 0.065\sigma' + 0.026\pi + 0.017\pi' + 0.0078$	0.546	0.095
$\log C/T = 0.035\pi - 0.079\pi' + 0.284\pi^2 + 0.276\pi'^2 - 0.075$	0.688	0.082
$\log C/T = 0.033\sigma - 0.168\sigma' + 0.054\pi - 0.420\pi' + 0.353\pi^2 + 0.555\pi'^2 - 0.169$	0.747	0.080

The observed values of log (C/T at maximum tolerated dose) in Table I are compared with those calculated from the last equation given above.

The results of linear regression analysis of the 13 Schiff bases of salicylaldehyde (R′ = 2-OH) are given below for comparison:

	r	s
$\log C/T = 0.016\sigma + 0.079$	0.163	0.097
$\log C/T = 0.025\pi + 0.089$	0.102	0.098
$\log C/T = 0.506\pi^2 + 0.018$	0.757	0.064
$\log C/T = 0.029\sigma + 0.062\pi + 0.067$	0.274	0.099
$\log C/T = -0.044\pi + 0.548\pi^2 + 0.013$	0.775	0.065
$\log C/T = 0.015\sigma - 0.022\pi + 0.533\pi^2 + 0.004$	0.786	0.067

Although the correlation coefficients are not remarkably good (r = 1 is perfect correlation), nevertheless

(1) E. M. Hodnett and W. Wilie, *Proc. Okla. Acad. Sci.*, **46**, 107 (1966).
(2) R. L. Reeves, *J. Org. Chem.*, **30**, 3129 (1965).
(3) "Protocols for Screening Chemical Agents and Natural Products Against Animal Tumors and Other Biological Systems," Cancer Chemotherapy National Service Center (CCNSC), *Cancer Chemother. Rep.*, **25**, 1 (1962), and as modified (Jan, 1966).
(4) C. Hansch and T. Fujita, *J. Amer. Chem. Soc.*, **86**, 1616 (1964); C. Hansch, *Accounts Chem. Res.*, **2**, 232 (1969).
(5) H. H. Jaffe, *Chem. Rev.*, **53**, 191 (1953).
(6) G. B. Barlin and D. D. Perrin, *Quart. Rev.*, **20**, 75 (1966).
(7) T. Fujita, J. Iwasa, and C. Hansch, *J. Amer. Chem. Soc.*, **86**, 5175 (1964).

TABLE I

ACTIVITIES OF AROMATIC SCHIFF BASES AGAINST INTRAMUSCULAR WALKER SARCOMA[a]

$R'C_6H_4CH{=}NC_6H_4R$

R	R'	Reference	Max tolerated dose,[b] mg/kg	T/C at the max tolerated dose[c]	$\Sigma\sigma$	$\Sigma\sigma'$	$\Sigma\pi$	$\Sigma\pi'$	Log (C/T at the max tolerated dose) Obsd	Log (C/T at the max tolerated dose) Calcd
2-CH_3	2-OH	*d*	400	0.58	0.29	1.22	0.68	−0.54	0.236	0.224
3-CH_3	2-OH	*e*	400	0.59	−0.07	1.22	0.51	−0.54	0.229	0.132
4-CH_3	2-OH	*f*	400	0.84	−0.17	1.22	0.52	−0.54	0.076	0.132
2-OH	2-OH	*g*	25	0.73	1.22	1.22	−0.54	−0.54	0.137	0.128
2-OH-5-Cl	2-OH	*h*	400	1.05	1.59	1.22	0.22	−0.54	−0.021	0.096
2-OCH_3	2-OH	*i*	400	0.12	0.12	1.22	−0.33	−0.54	0.055	0.038
4-OCH_3	2-OH	*j*	400	0.97	−0.27	1.22	−0.04	−0.54	0.013	0.004
2-NO_2	2-OH	*k*	400	0.91	1.99	1.22	−0.23	−0.54	0.041	0.086
4-NO_2	2-OH	*l*	400	1.01	0.78	1.22	0.24	−0.54	−0.004	0.074
2-OH-4-NO_2	2-OH	*m*	400	0.75	2.00	1.22	−0.30	−0.54	0.125	0.096
2-OH-5-NO_2	2-OH	*m*	400	0.62	1.93	1.22	−0.43	−0.54	0.208	0.120
4-$N(CH_3)_2$	2-OH	*n*	500	1.10	−0.83	1.22	0.10	−0.54	−0.041	−0.004
4-CO_2H	2-OH	*e*	400	0.77	0.41	1.22	0.12	−0.54	0.114	0.040
2-OH-5-Cl	H	*o*	400	1.17	1.59	0.00	0.22	0.00	−0.68	−0.088
2-OH-5-NO_2	H	*o*	400	1.21	1.93	0.00	−0.43	0.00	−0.083	−0.063
4-OH	4-OCH_3	*m*	400	1.43	−0.37	−0.27	−0.61	−0.04	−0.155	−0.020
2-OH-5-Cl	4-OCH_3	*m*	400	0.87	1.59	−0.27	0.22	−0.04	0.060	−0.024
2-OH-5-NO_2	4-OCH_3	*g*	400	0.85	1.93	−0.27	−0.43	−0.04	0.070	0.000
2-OH-4-NO_2	4-OCH_3	*m*	400	1.11	2.00	−0.83	−0.30	−0.04	−0.045	−0.024
2-OH	4-$N(CH_3)_2$	*g*	400	1.17	1.22	−0.83	−0.54	0.33	−0.068	0.006
3-OH	4-$N(CH_3)_2$	*m*	400	0.83	0.10	−0.83	−0.49	0.33	0.081	−0.047
4-OH	4-$N(CH_3)_2$	*p*	400	0.98	−0.37	−0.83	−0.61	0.33	0.009	−0.022
2-OH-5-Cl	4-$N(CH_3)_2$	*q*	400	1.17	1.59	−0.83	0.22	0.33	−0.068	−0.026
2-OH-5-NO_2	4-$N(CH_3)_2$	*g*	400	1.11	1.93	−0.83	0.43	0.33	−0.045	−0.002

[a] The screening data were supplied through the kindness of Dr. Harry B. Wood, Jr., of the Cancer Chemotherapy National Service Center, National Institutes of Health, Bethesda, Md. Assays were performed according to CCNSC specifications in *Cancer Chemother. Rep.*, **25**, 1 (1962). [b] One dose daily for 4 days. [c] Effectiveness against intramuscular Walker sarcoma of the rat is measured by weights of tumors of treated rats (T) compared with the tumors of control rats (C); the value of T/C must be 0.53 or less for significant activity. [d] A. Hantzsch and O. Schwab, *Ber.*, **34**, 833 (1901). [e] A. Senier and F. G. Shepheard, *J. Chem. Soc.*, **95**, 1943 (1909). [f] P. I. Ittyerah and K. C. Pandya, *J. Indian Chem. Soc.*, **30**, 717 (1953). [g] A. W. Baker and A. T. Shulgin, *J. Amer. Chem. Soc.*, **81**, 1523 (1959). [h] J. Argauer and C. E. White, *Anal. Chem.*, **36**, 2141 (1964). [i] A. Senier, F. G. Shepheard, and R. Clarke, *J. Chem. Soc.*, **101**, 1950 (1912). [j] A. Hantzsch and E. Wechsler, *Justus Liebigs Ann. Chem.*, **325**, 226 (1902). [k] Mp 113.5–114°. *Anal.* ($C_{13}H_{10}N_2O_3$) C, H, N. [l] F. G. Pope, *J. Chem. Soc.*, **93**, 532 (1908). [m] E. M. Hodnett and C. Capshew, *Proc. Okla. Acad. Science*, in press. [n] H. A. Torrey, *Amer. Chem. J.*, **34**, 474 (1905). [o] L. C. Raiford and J. Linsk, *J. Amer. Chem. Soc.*, **67**, 878 (1945). [p] G. Smets and A. Delvau, *Bull. Chim. Belges*, **56**, 106 (1947). [q] L. Muslin, W. Roth, and H. Erlenmeyer, *Helv. Chim. Acta*, **36**, 886 (1953).

TABLE II

CONTRIBUTIONS OF SUBSTITUENTS TO ACTIVITY AGAINST INTRAMUSCULAR WALKER SARCOMA[a]

$R'C_6H_4CH{=}NC_6H_4R$

R'	n[b]	C/T at maximum tolerated dose	R	n[b]	C/T at maximum tolerated dose
2-H	11	−0.272	2-H	9	−0.178
2-OH	13	0.230	2-CH_3	1	0.515
			2-OH	12	0.105
4-H	15	−0.054	2-OCH_3	1	−0.065
4-OCH_3	4	0.034	2-NO_2	1	−0.105
4-$N(CH_3)_2$	5	0.136	3-H	22	−0.051
			3-CH_3	1	0.612
			3-OH	1	0.521
			4-H	15	−0.024
			4-CH_3	1	0.139
			4-OH	2	0.161
			4-OCH_3	1	−0.021
			4-NO_2	3	−0.061
			4-$N(CH_3)_2$	1	−0.142
			4-CO_2H	1	0.249
			5-H	16	−0.011
			5-NO_2	4	0.111
			5-Cl	4	−0.066

[a] The overall C/T at maximum tolerated dose is 1.116. [b] The number of compounds with each substituent.

some conclusions may be drawn from the results of this study.

Better correlation of antitumor effects is obtained with substituents of the aldehyde moiety than with those of the amine group. This fact may indicate that the aldehyde group is more important to the antitumor value than the amine group is.

Results of the correlation of antitumor activity of the Schiff bases from salicylaldehyde are somewhat better than those of the Schiff bases from all the aldehydes, but not as much as might be expected from the greater similarity of the Schiff bases from salicylaldehyde. The best correlation is obtained with π^2 values; the biological meaning of this is not clear. Substituents with large values of π are desirable for antitumor activity.

Increased antitumor activity is related to a large positive value of σ; electron withdrawal from the ring by the substituent group is desirable for antitumor activity. This fact may indicate that molecules containing such electron-withdrawing groups attach to receptor sites better or that stability of the Schiff base (increased by electron-withdrawing groups) is a desirable factor for antitumor activity of the Schiff bases.

Increased antitumor activity is related to large posi-

tive values of π. Substituents which contribute to the lipophilic nature of the molecule should therefore be sought.

These data were also analyzed by the method of Free and Wilson.[8] By subjecting our data to multiple linear regression analysis[9] the contribution of each substituent to the antitumor activity of the molecule was determined (Table II). In order to estimate the value of $C \cdot T$ at the maximum tolerated dose for each compound, the contribution of each substituent in the 5 positions of the two rings is added to the base activity, 1.116. Since $C \cdot T$ at the maximum tolerated dose should be as large as possible for good antitumor activity the results indicate the desirability of having in the amine moiety, Me groups at the 2, 3, and 4 positions, OH groups in the 2, 3, and 4 positions, CO_2H at the 4 position, and NO_2 in the 5 position. Substitution in the aldehyde moiety is desirable especially for OH in the 2 position; in this case the substituent effect of the 2-OH is very certain since it is substantiated by 13 compounds.

Acknowledgments.—Grateful acknowledgment is made of the valuable assistance of Paul Mooney, Charles Capshew, and Joyce Wan in the preparation of these compounds, the staff of the Oklahoma State University Computing Center in making the correlations, and the staff of the Research Foundation of Oklahoma State University in preparing this report.

(8) S. M. Free, Jr., and J. W. Wilson, *J. Med. Chem.*, **7**, 395 (1964).

(9) The computer program used for this purpose was a modified version of the multiple regression program contained in IBM's SYSTEM/360 Scientific Subroutine Package. This program was maintained on tape and required only a few seconds of computation time on the IBM 360/50 computer.

Antineoplastic Research. 1. Pyrazole and Pyrimidine Derivatives of Dehydrocycloheximide Analogs[1]

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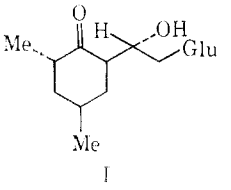
Cycloheximide[2] (I), a product of *Streptomyces griseus* cultures, is well known for its ability to inhibit protein biosynthesis.[3] This biological action as well as that of emetine, anisomycin, and others is attributed to the stereochemical relationships between the N and O atoms of the molecule.[4] It has also been demonstrated that this relationship can be affected by a change in the substitution pattern or stereochemistry at many of the other positions in the molecule.[5]



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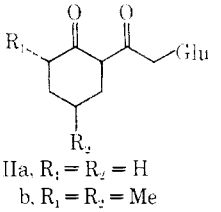
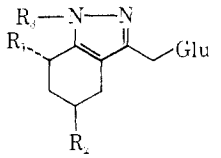
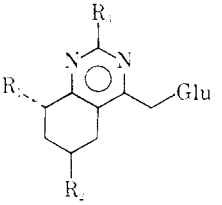


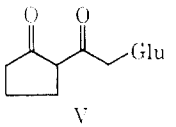
IIa, $R_1 = R_2 = H$
b, $R_1 = R_2 = Me$
c, $R_1 = H$; $R_2 = Me$
d, $R_1 = Me$; $R_2 = H$



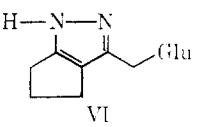
IIIa, $R_1 = R_2 = R_3 = H$
b, $R_1 = R_2 = H$; $R_3 = C_6H_5$
c, $R_1 = R_2 = Me$; $R_3 = H$
d, $R_1 = H$; $R_2 = Me$; $R_3 = H$
e, $R_1 = H$; $R_2 = Me$; $R_3 = C_6H_5$
f, $R_1 = Me$; $R_2 = R_3 = H$



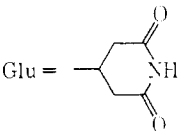
IVa, $R_1 = R_2 = H$; $R_3 = NH_2$
b, $R_1 = R_2 = H$; $R_3 = SH$
c, $R_1 = R_2 = Me$; $R_3 = NH_2$
d, $R_1 = Me$; $R_2 = H$; $R_3 = NH_2$



V



VI



In order to establish further points about the structure–activity relationships of these molecules we have undertaken a program directed toward the synthesis of several modified glutarimide antibiotics. During the course of these studies we had occasion to prepare a a few dehydrocycloheximide type compounds II which could also be converted into a variety of heterocyclic compounds through the 1,3-diketone system. Even though we would be destroying the essential stereochemical features, the combination of the salient parts of cycloheximide with heterocyclic systems appeared attractive for its biological potential.

The required 1,3-diketones II and V were prepared from the enamine of the appropriately substituted ketone and the acid chloride of 2-(β-glutarimido)acetic acid according to the acylation procedure developed by Johnson, *et al.*,[6] in their elegant total synthesis of cycloheximide. Though Struck and coworkers[7] had prepared diketones IIa,c,d by a similar method, we found the Johnson modifications generally gave much better yields. Dehydrocycloheximide (IIb) was more easily prepared by the Jones' oxidation of cycloheximide (I) at ambient temperature.[8]

The pyrazole derivatives III and VI were easily formed by the action of hydrazine and phenylhydrazine on the 1,3-diketones. Although we have indicated the

(1) This work was aided by Grant T-474 from the American Cancer Society and Grant 67-16 from its Illinois Division.

(2) Actidione®.

(3) For recent reports see B. S. Baliga, A. W. Pronczuk, and H. N. Munro, *J. Biol. Chem.*, **244**, 4480 (1969); A. Geller, F. Robustelli, S. H. Barondes, H. D. Cohen, and M. E. Jarvik, *Psychopharmacologia*, **14**, 371 (1969); J. A. Burdman and L. J. Journey, *J. Neurochem.*, **16**, 493 (1969); S. D. J. Yeh and M. Shils, *Biochem. Pharmacol.*, **18**, 1919 (1969).

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(5) H. D. Sisler and M. R. Siegel, in "Antibiotics," Vol. I, D. Gottlieb and P. D. Shaw, Ed., Springer-Verlag, New York, N. Y., 1967, p 283.

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(7) R. F. Struck, H. S. Schaeffer, C. A. Krauth, R. T. Kemp, Y. F. Shealy, and J. A. Montgomery, *J. Med. Chem.*, **7**, 646 (1964).

(8) Yields were generally 70–80% decidedly better than the CrO_3–HOAc method originally used by E. C. Kornfield, R. G. Jones, and T. V. Parke [*J. Amer. Chem. Soc.*, **71**, 150 (1949)].