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Quantitative Structure-Activity Relationships in Anticancer Agents. Activity of Selected Nitrosoureas against a Solid Tumor, the Lewis Lung Carcinoma†

John A. Montgomery,* Joseph G. Mayo,

Kettering-Meyer Laboratory, Southern Research Institute, Birmingham, Alabama 35205

and Corwin Hansch

Department of Chemistry, Pomona College, Claremont, California 91711. *Received November 5, 1973*

Fourteen nitrosoureas, varying in log P values from 4.51 to −2.21, have been evaluated at a series of dose levels for their ability to delay the growth of a solid tumor, the Lewis lung carcinoma, in mice. A reasonable correlation of activity with partition coefficients was obtained. The ideal log P value for nitrosoureas that inhibit the Lewis lung carcinoma should lie between −0.20 and +1.34.

Recent studies have shown that despite the great complexity of the problem, it is possible to formulate quantitative structure-activity relationships (QSAR) for congeneric sets of antitumor agents.[1] In particular, it has been demonstrated that antitumor activity depends heavily on the relative lipophilic character of drugs as operationally defined by log P where P is the octanol-water partition coefficient. A large amount of evidence is now in hand showing that, other factors being constant, one characteristically finds that biological response to a very wide variety of drugs shows a parabolic dependence on lipophilic character.[2]

In the present study 14 nitrosoureas,[3-5] varying in log P values from 4.51 to −2.21, were selected for a pilot study against the Lewis lung carcinoma. The primary object of the study was to further explore the utility of log P in the design of antitumor agents.

Method

A review of the data at hand when this experiment was undertaken indicated that early treatment would probably give the best results for this type of study. Consequently, a series of doses of each drug was administered 24 hr after tumor implantation (Table I) (see ref 6 for detailed protocol).

Semilog plots (illustrated by Figure 1) were prepared of the growth of the control tumors *vs.* the treated tumors, and the maximum delay in tumor growth during the first 20 days posttreatment was determined from these plots for each level of drug. From these data, plots of delays in days of tumor growth, a measure of the number of carcinoma cells killed, were prepared (illustrated by Figure 2). The molar concentration, C, required to produce a delay in tumor growth of 4 days was arbitrarily selected as the basis of comparison for this set of drugs. However, it must be borne in mind that some of these compounds (see Table I) caused cures of the Lewis lung carcinoma at some dose levels, and these delays in tumor growth would have to be considered infinite and are, therefore, not included in the calculations. These drugs, then, are more active against this tumor than these comparisons would indicate.

Many of the log P values of Table II have been previously reported.[1] The others were determined by Dr. William J. Haggerty of the Midwest Research Institute under Contract 69-2113 with DCT, NCI, National Institutes of Health, except the values for NSC-175377 and NSC-176960, which were calculated.

Results and Discussion

From the data in Table II, eq 1-3 have been formulated *via* the method of least squares. In the above equations, n represents the number of data points, r the correlation coefficient, and s the standard deviation. The figures in parentheses are the 95% confidence limits.

	n	r	s	
$\log 1/C = -0.06\ (\pm 0.11) \log P + 0.96\ (\pm 0.27)$	13	0.321	0.400	(1)
$\log 1/C = -0.082\ (\pm 0.05)\ (\log P)^2 + 0.14\ (\pm 0.15) \log P + 1.23\ (\pm 0.26)$	13	0.765	0.285	(2)
$\log 1/C = -0.081\ (\pm 0.11)\ (\log P)^2 + 0.13\ (\pm 0.44) \log P + 1.25\ (\pm 0.44)$	8	0.860	0.250	(3)

To formulate eq 1 and 2, all of the active congeners of Table II were employed. Although eq 2 is not as sharp a correlation as others which have been obtained with antitumor drugs,[1] it is statistically quite significant ($F_{2,10}$ = 7.1; $F_{2,10,\alpha 0.025}$ = 5.5). From eq 2, the ideal log P is found to be 0.83 (−0.20 to 1.34). This is shown graphically in Figure 3 which suggests that the best region to prospect for active drugs, steric and electronic effects being neglected, is in the log P range of −1.0 to 2.0. Since previous results showed[1] that nitrosoureas with negative log P values were less toxic (LD_{10}), it would seem that the re-

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Table I. Selected Nitrosoureas *vs.* Early Lewis Lung Carcinoma Data (R_x = Day 1 Only, ip)

NSC no.	Dosage, mg/kg	Max delay in tumor growth, days	Median life span			Tumor-free survivors/ total
			Control	Treated	% ILS	
88104	57	4.0	28.5	39.5	+38	0/10
	38	4.0	28.5	41.0	+43	0/10
	25	4.0	28.5	28.0	−2	0/10
	17	1.4	28.5	32.0	+12	0/10
	11	0.2	28.5	32.5	+14	0/10
93372	152	6.9	25.0	31.5	+26	0/10
	101	2.3	25.0	32.0	+28	0/10
	67	1.9	25.0	33.0	+32	0/10
	45	0	25.0	25.5	+2	0/10
	30	0	25.0	34.5	+38	0/10
95439	315	18.5	28.5	34.0	+19	0/10
	210	5.2	28.5	35.5	+24	0/10
	140	1.2	28.5	34.0	+19	0/10
95441	36	>11.3	28.5	46.0	>+61	3/10
	24	6.8	28.5	28.0	−2	0/10
	16	2.5	28.5	28.0	−2	0/10
	11	2.3	28.5	27.5	−5	0/10
	7	1.8	28.5	31.0	+8	0/10
95441	36	>1.3	25.0	29.0	>+16	3/10
	24	>7.0	25.0	41.0	>+64	1/10
	16	1.8	25.0	35.5	+42	0/10
	11	−0.5	25.0	29.0	+16	0/10
	7	−1.5	25.0	30.5	+22	0/10
95466	26	>10.6	25.0	32.0	>+28	1/10
	17	3.4	25.0	32.0	+28	0/10
	11	1.4	25.0	33.0	+32	0/10
	7	0	25.0	33.5	+34	0/10
	5	0	25.0	22.5	−10	0/10
106767	32	>14	25.0	24.0	>−4	2/10
	21	>8	25.0	29.5	>+18	2/10
	14	1.8	25.0	26.0	+4	0/10
	9	0	25.0	30.5	+22	0/10
128303	23	7.8	25.0	39.0	+56	0/10
	15	3.8	25.0	35.5	+42	0/10
	10	2.0	25.0	31.0	+24	0/10
	7	0	25.0	29.5	+18	0/10
	5	0	25.0	29.0	+16	0/10
129968	85	4.4	33.0	33.0	0	0/10
	57	2.8	33.0	32.0	−4	0/9
	38	1.6	33.0	34.0	+3	0/10
	25	0	33.0	34.0	+3	0/10
153174	26	6.0	25.0	26.0	+4	0/10
	17	6.0	25.0	31.0	+24	0/10
	11	2.5	25.0	29.0	+16	0/10
	7	0.5	25.0	28.0	+12	0/10
	5	0	25.0	29.5	+18	0/10
	3	0	25.0	25.0	0	0/10
153175	27	>10.3	25.0	33.0	>+32	3/10
	18	>5	25.0	31.5	>+26	2/10
	12	3	25.0	27.5	+10	0/10
	8	1	25.0	31.5	+26	0/10
	5	−0.5	25.0	21.0	−16	0/10
	3	−1.3	25.0	29.5	+18	0/10
163478	183	7.5	25.0	40.0	+60	0/10
	122	2.5	25.0	24.0	−4	0/10
	81	−1.3	25.0	25.0	0	0/10
175377	26	>10.5	30.5	41.0	>+34	4/10
	18	2.5	30.5	27.0	−11	0/10
176960	82	6.5	16.5	21.0	+27	0/10
	55	1.8	16.5	16.5	0	0/10
	36	1.5	16.5	15.5	−7	0/10

gion −1.0 to 0.0 is most worthy of more intensive exploration.

The compounds upon which eq 1 and 2 are based consist of a set of neutral and ionized (derivatives containing a COOH) molecules. Almost no experience has been obtained with such mixed sets of congeners. Log P for ion pairs depends heavily upon the nature of the counterion. To obtain octanol–water log P values, sodium salts are partitioned. However, in the living organism other cations (*e.g.*, an amino acid) might serve as the counterion. Since the log P values for neutral molecules are not very sensitive to ionic strength or the type of ions present while ionized compounds are, we are not yet in a position to know how well log P values will model such a mixed set of congeners. The present study is encouraging in that it suggests that reasonable results can be obtained. Omitting the five derivatives containing a COOH function, eq 3 is obtained. While this is a sharper correlation in terms of r and s, the constants in eq 3 are identical with those of eq 2. Hence, both equations yield the same ideal value for

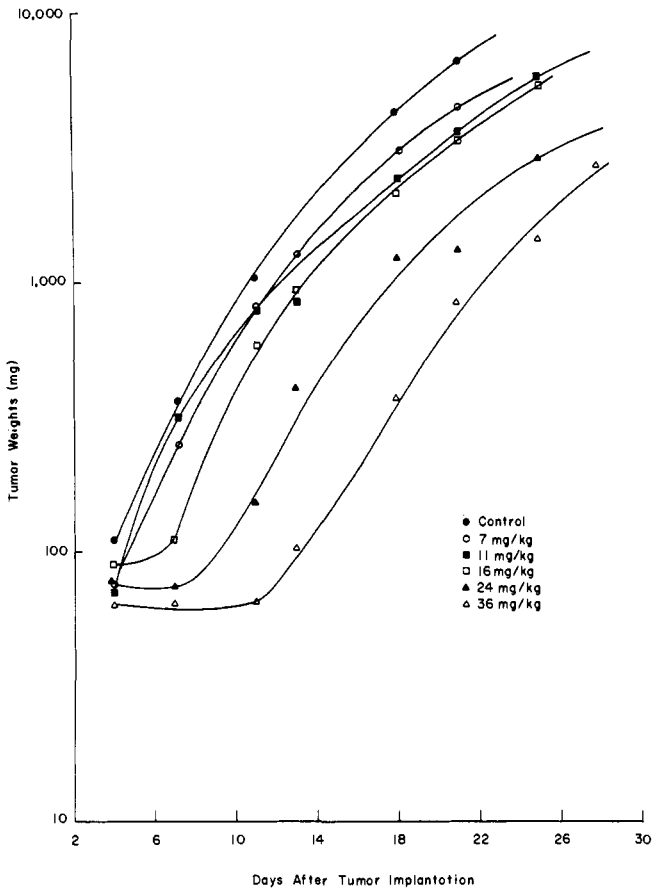


Figure 1. Inhibition of the Lewis lung carcinoma by 1-(2-chloroethyl)-3-(4-*trans*-methylcyclohexyl)-1-nitrosourea (R_x = day 1 only, ip).

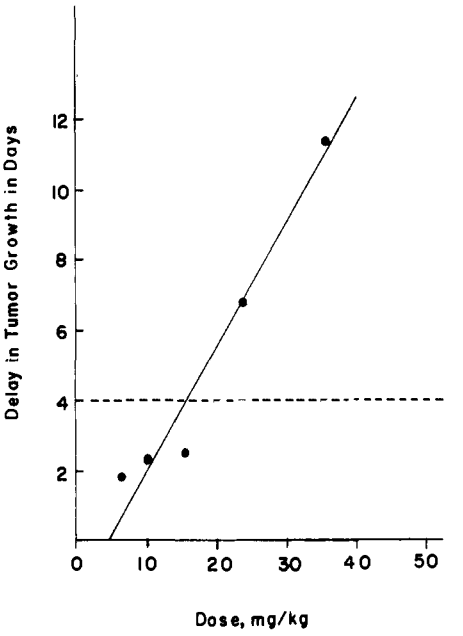


Figure 2. Delay in tumor growth caused by 1-(2-chloroethyl)-3-(4-*trans*-methylcyclohexyl)-1-nitrosourea (R_x = day 1 only, ip).

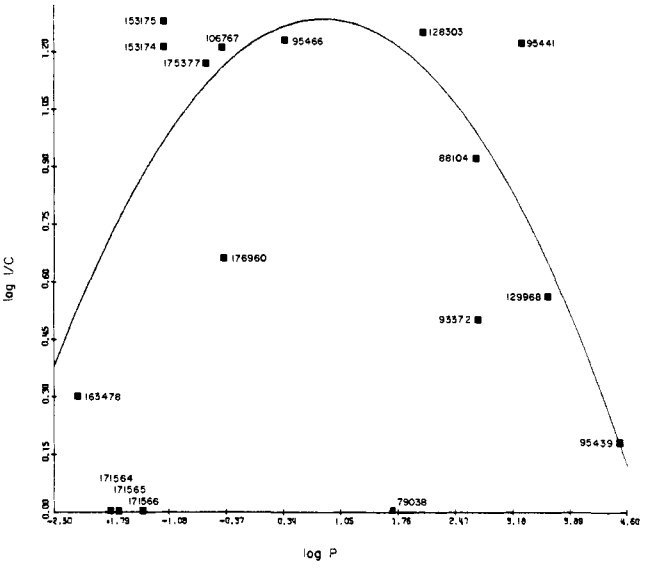


Figure 3. Selected nitrosoureas *vs.* early Lewis lung carcinoma (R_x = day 1 only, ip).

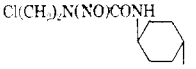
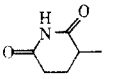
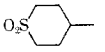
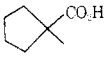
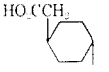
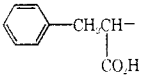
lipophilic character (log P_0). This result could be taken to mean that the scatter in Figure 3 is not primarily due to poor log P values, but to other factors.

We are not able to parameterize the electronic or steric effect of substituents on the NH group. The fact that nitrosoureas with phenyl groups attached directly to nitrogen are completely inactive despite reasonable log P values might be due to the delocalization of the lone pair electrons of the NH unit. *N*-[(2-Chloroethyl)nitrosocarbamoyl]-DL-alanine (NSC-171564), 2-methyl-*N*-[(2-chloroethyl)nitrosocarbamoyl]-DL-alanine (NSC-171565), and 1-[3-(2-chloroethyl)-3-nitrosoureido]cyclopentanecarboxylic acid (NSC-171566), which are completely inactive, and *N*-[(2-chloroethyl)nitrosocarbamoyl]-3-phenyl-DL-alanine (NSC-176960), which is considerably less active than expected, all contain a COOH group (which would be ionized at physiological pH) only one carbon removed from the NH moiety. This same situation occurs in *N*-[2-chloroethyl)nitrosocarbamoyl]-L(+)-glutamic acid (NSC-163478), a dicarboxylic acid that is also much less active than predicted by eq 2. The fact that congeners with the COOH well removed from the NH, 4-[3-(2-chloroethyl)-3-nitrosoureido]-*cis*- and -*trans*-cyclohexanecarboxylic acids (NSC-153174 and NSC-153175) and 4-[3-(2-chloroethyl)-3-nitrosoureido]-*trans*-cyclohexaneacetic acid (NSC-175377), are well predicted by eq 2 indicates that the COOH function *per se* does not destroy activity.

For cyclohexyl, $\sigma^* = -0.15$ while for $-C_6H_5$, $\sigma^* = 0.60$. Actually, electron withdrawal by phenyl attached directly to N may be greater than indicated by its σ^* value. In any case, the phenyl group is strongly electron withdrawing, while cyclohexyl is electron releasing. This does suggest that high electron density on nitrogen is significant for activity. If this surmise is correct, then greater activity might be obtained by increasing the electron density on NH. Unfortunately, there is almost nothing more electron releasing than a secondary carbon such as cyclohexyl. A tertiary carbon such as *tert*-butyl is more electron releasing; however, this advantage might be offset by its greater steric effect. It would be interesting to test $FCH_2CH_2N(NO)CONHC(Me)_3$; the calculated log P for this molecule is about 1.4. Neglecting electronic and steric factors, its calculated log $1/C$ from eq 2 is 1.18. If the steric effects of the *tert*-butyl group are not adverse (they could be favorable), one should find log $1/C > 1.18$. Ideally, one would like log P to be considerably lower than 1.4; this would be difficult to achieve by modifying the *tert*-butyl function, but it might be possible by means of a more hydrophilic and electron-withdrawing group than F. An interesting possibility might be $NCCH_2CH_2N(NO)CONHC(Me)_3$.

Two compounds with the COOH adjacent to the NH moiety are NSC-163478 and NSC-176960. Since these

Table II. QSAR. Selected Nitrosoureas *vs.* Early Lewis Lung Carcinoma (R_x = Day 1 Only, ip)

NSC no.	RNHCON(NO)$(CH_2)_2$Cl, R	Mol wt	*C*, mg/kg	*C*, mmol/kg	1/*C*	Log 1/*C*	Log *P*
79038	NC—C_6H_4–	252.7		Inactive			1.66
88104		268.1	32.0	0.119	8.38	0.923	2.73
93372		383.2	120	0.313	3.19	0.504	2.74
95439		289.8	190	0.656	1.53	0.183	4.51
95441		247.7	15.0	0.0605	16.5	1.22	3.30
95466		262.7	15.5	0.0590	16.95	1.23	0.37
106767		267.3	16.3	0.0610	16.4	1.21	−0.41
128303		269.8	15.0	0.0556	18.0	1.255	2.08
129968		285.8	78.0	0.273	3.66	0.564	3.62
153174		277.7	17.0	0.0612	16.3	1.21	−1.14
153175		277.7	14.5	0.0522	19.2	1.28	−1.14
163478	$HO_2C(CH_2)_2(HO_2C)CH$–	281.7	140	0.497	2.01	0.303	−2.21
171564	$HO_2C(Me)CH$–	241.5		Inactive			−1.8
171565	$HO_2C(Me)_2C$–	255.5		Inactive			−1.7
171566		281.5		Inactive			−1.4
175377		291.7	19.5	0.0668	14.96	1.175	−0.61
176960		285.7	63	0.2205	4.53	0.657	−0.40

$$\log 1/C = -0.061\ (\pm 0.04)\ (\log P)^2 + 0.038\ (\pm 0.12)\ \log P - 0.62\ (\pm 0.42)\ D + 1.31\ (\pm 0.19) \quad (4)$$

n = 13, r = 0.904, s = 0.199

compounds are active, we can use a dummy parameter to account for such a structure. Assigning D = 1.00 for the above two compounds and D = 0 for all others, eq 4 can be derived. Log P_0 for eq 4 is 0.31 (−1.8 to 0.9) which is close to that obtained from eq 2. While eq 4 is significantly better than eq 2 ($F_{1,9}$ = 11.4; $F_{1,9,\alpha 0.01}$ = 10.6), one cannot place any real confidence in the coefficient of D since it rests on only two data points. Nevertheless, the negative coefficient with D gives some idea of the deleterious effect of the COOH function.

The data presented here indicate that delay in the growth of Lewis lung carcinoma following a single dose of drug administered 24 hr after implantation of the tumor, which is a good measure of cell kill, can be used as an end point for a QSAR for nitrosoureas acting against a solid tumor. At the same time, increase in life span (see Table I), which depends on effects on the rate of metastasis and other less well-understood factors as well as cell kill in the primary tumor, does not appear to be a satisfactory parameter for this type of study.

References

(1) C. Hansch, N. Smith, R. Engle, and H. Wood, *Cancer Chemother. Rep.*, **56**, 443 (1972).
(2) C. Hansch and J. M. Clayton, *J. Pharm. Sci.*, **62**, 1 (1973).
(3) T. P. Johnston, G. S. McCaleb, P. S. Opliger, and J. A. Montgomery, *J. Med. Chem.*, **9**, 892 (1966).
(4) T. P. Johnston, G. S. McCaleb, P. S. Obliger, W. R. Laster, and J. A. Montgomery, *J. Med. Chem.*, **14**, 600 (1971).
(5) T. P. Johnston, G. S. McCaleb, and J. A. Montgomery, to be submitted for publication.
(6) R. I. Geran, N. H. Greenberg, M. M. Macdonald, A. M. Schumacher, and B. J. Abbott, *Cancer Chemother. Rep., Part 3*, **3** (2), 13 (1972).