Quantitative Structure–Activity Relationships of Purines II: Prediction of Activity against Adenocarcinoma CA755 and Toxicity in Mice

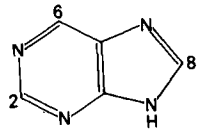
ZOHAR NEIMAN and FRANK R. QUINN ˣ

Received June 11, 1981, from the *Laboratory of Medicinal Chemistry and Biology, Division of Cancer Treatment, National Cancer Institute, National Institutes of Health, Bethesda, MD 20205.* Accepted for publication September 16, 1981.

Abstract □ Quantitative structure–activity relationships (QSAR) were derived for a number of 2,6-mono- and disubstituted purines. The derived equations relate the anticancer activity in murine Adenocarcinoma CA755 to the molar refractivity of substituents at position 2 and electron-withdrawing effects of substituents at position 6. A QSAR was also derived for the acute toxicity (LD_{50}) of substituents at position 6. The results suggest that toxicity is relatively independent of the nature of the substituent.

Keyphrases □ Anticancer activity—of purines, prediction of activity against Adenocarcinoma CA755 and toxicity in mice □ Purines—anticancer activity, prediction of activity against Adenocarcinoma CA755 and toxicity in mice, structure–activity relationship □ Structure–activity relationships—of purines, prediction of activity against Adenocarcinoma CA755 and toxicity in mice

Purine (I) and purine nucleosides comprise a very important class of potential anticancer agents (1). Many hundreds of purine analogs have been synthesized (2) and tested for their anticancer properties. Of the purine bases, only mercaptopurine and thioguanine have found general usage in the clinical treatment of cancer (3).



In a previous study (4) it was demonstrated that quantitative structure–activity relationships (QSAR) were possible with purines in spite of the fact that the purine ring system is not typically aromatic (5). In the present study quantitative structure–activity correlations were derived for the *in vivo* activity and toxicity of a number of 2- and 6-substituted purines against the murine solid tumor Adenocarcinoma 755(CA755).

It has been pointed out (6) that CA755 is a test system that is artificially sensitive to purines. Under the protocols for testing drugs against CA755 (7) many purines prove to be impressively carcinostatic and sometimes carcinolytic. However, these test conditions, in which the drug is administered 24 hr after tumor implantation, reflect treatment during early stages of the disease. It has been shown (6) that, if treatment is delayed until the disease is well-advanced, a situation more typical of that encountered in humans, these purines are not as effective. The principal value in using data from a sensitive system such as CA755 is that the effect of subtle structural changes on the activity may be easily discerned. The ultimate effectiveness of any analog against other animal or human tumors would still have to be determined.

EXPERIMENTAL

The substituted constants employed in this study included the hydrophobic parameter π and the Swain-Lupton field and resonance constants $\mathcal{F}$ and $\mathcal{R}$ (8). The molar refractivity (MR) was used as a measure of steric bulk (8). The physicochemical parameters which appear in the correlations are given in Table I. Substituent constants were taken from the recent literature (9). Values of π not available from the literature were calculated by the additive method (10); MR values were calculated from Vogel's bond refraction values (11).

It should be noted that the π values used in this study were determined in an aromatic system, *i.e.*, from benzene derivatives. Purine, on the other hand, is a nitrogen heterocycle and substituents on the purine ring may undergo various perturbations due to inter- and intramolecular associations with the ring nitrogens. It is quite possible that π values for purine substituents would differ considerably from those of their benzene analogs. The major deviations from standard π values would be expected for substituents at positions 2 and 8, both of which are adjacent to two-ring nitrogen atoms. The 6 position, on the other hand, may be similarly af-

Table I—Physicochemical Parameters

Substituent	π	$\mathcal{R}$	MR
Hydrogen	0	0	0.103
Methyl	0.56	−0.13	0.565
Cyano	−0.57	0.19	0.633
Chloro	0.71	−0.15	0.603
Bromo	0.86	−0.17	0.888
Iodo	1.12	−0.19	1.394
Methoxy	−0.02	−0.51	0.787
Ethoxy	0.38	−0.44	1.247
n-Propoxy	1.05	−0.45	1.706
Amino	−1.23	−0.68	0.542
Methylamino	−0.47	−0.74	1.033
Dimethylamino	0.18	−0.92	1.555
Trimethylammonium	−5.96	0	—
Benzamido	0.49	−0.27	3.464
Hydrazino	−0.88	−0.71	0.844
Hydroxylamino	−1.34	−0.40	0.722
Methylthio	0.61	—	1.382
Ethylthio	1.07	—	1.842
n-Propylthio	1.57	—	2.357
n-Butylthio	2.07	—	2.819
iso-Butylthio	2.16	—	2.819
sec-Butylthio	2.16	—	2.819
2′-Propynylthio	1.01	—	2.141
Cyanomethylthio	−0.23	—	1.869
Cyclopentylthio	2.25	—	2.994
Phenylthio	2.32	—	3.429
Thiocyanate	0.41	—	1.340
2-Imidazolinthio	0.63	—	2.741
1′-Methyl-4′-nitroimidazol-5′-ylthio	0.27	—	3.582
Benzylthio	2.57	—	3.793
3,4-Dimethylbenzylthio	3.57	—	4.680
o-Chlorobenzylthio	3.53	—	3.484
o-Fluorobenzylthio	2.96	—	3.780
o-Nitrobenzylthio	2.29	—	4.410
m-Chlorobenzylthio	3.28	—	4.234
p-Fluorobenzylthio	2.96	—	3.780
2′-Pyridylmethylthio	1.16	—	3.633
Methylsulfonyl	−1.63	0.22	1.349
Ethylsulfonyl	−1.13	—	1.814
n-Propylsulfonyl	−0.63	—	2.279
n-Butylsulfonyl	−0.13	—	2.743
Fluorosulfonyl	0.05	0.22	0.865
Sulfonamide	−1.82	0.19	1.228
N-Methylsulfonamide	−1.32	—	1.712
N-*n*-Propylsulfonamide	−0.32	—	2.643
N-iso-Butylsulfonamide	0.23	—	3.282
N′-3′-Methoxypropylsulfonamide	−0.34	—	3.285
N-2′-Ethoxyethylsulfonamide	−0.44	—	3.242
N-Benzylsulfonamide	0.19	—	4.053

0022-3549/82/0600-0618$01.00/0

Table II—Observed and Predicted CA755 Activity of Mono- and Disubstituted Purines

Number	Compound	log(1/C)[a] Observed	log(1/C)[a] Predicted	|Δlog(1/C)|
I	Purine	Inactive	4.18	—
II	6-Chloro	4.23	4.08	0.15
III	6-Bromo	4.50	4.06	0.44
IV	6-Iodo	4.52	4.03	0.49
V	6-Methoxy	3.00	3.65	0.65
VI	6-*n*-Propoxy	3.42	3.73	0.31
VII	6-Hydrazino	3.22	3.42	0.20
VIII	6-Trimethyl-ammonio	4.34	4.26	0.08
IX	6-Methylthio	4.51	4.04	0.47
X	6-Ethylthio	4.45	4.04	0.41
XI	6-Methylsulfonyl	4.04	4.52	0.48
XII	6-Sulfonamide	4.26	4.48	0.22
XIII	2-Methyl-6-amino	3.23	3.19	0.04
XIV	2-Chloro-6-methylamino	3.37	3.10	0.27
XV	2-Bromo-6-amino	2.56	3.04	0.48
XVI	2-Amino-6-chloro	4.05	3.82	0.23
XVII	2-Amino-6-bromo	3.91	3.80	0.11
XVIII	2-Amino-6-iodo	3.91	3.78	0.13
XIX	2-Amino-6-methyl-sulfonyl	4.04	4.26	0.22
XX	2-Dimethylamino-6-methylamino	3.12	2.65	0.47
XXI	2,6-Bishydrazino	2.77	3.02	0.25
XXII	2,6-Bis(methyl sulfonyl)	3.64	3.63	0.01
XXIII	2-Flurosulfonyl-6-chloro	3.18	3.67	0.49

[a] *C* is the concentration (moles/kg) which produced a tumor weight regression = 80%. [b] From Eq. 1.

fected but to a lesser extent since substituents are α to only one-ring nitrogen. This effect was tested by examining the π values of a number of 2-substituted pyridines in comparison with normal π values. Substituents were CN, Cl, Br, $COCH_3$, CH_3, C_2H_5, CH_3O, NH_2, $NHCOCH_3$ and NO_2. The following relationship was found:

$$\pi \text{ pyridines} = 0.38(\pm 0.17) + 0.49(\pm 0.22)\ \pi \text{ aromatics}$$

$$n = 10;\ r = 0.876;\ s = 0.227;\ F_{1,9} = 27.06\ (p < 0.001)$$

This relationship which was derived for some widely diverse substituents suggests that normal aromatic π values may be employed at position 6 with relative safety.

Antitumor data (Table II) were collected by the Drug Evaluation Branch, National Cancer Institute (NCI), according to the protocols (7) mentioned previously. A tumor fragment was implanted subcutaneously and treatment was initiated 24 hr later. The drug was administered daily for 11 consecutive days (QD1–11). On the twelfth day the animals were sacrificed and the tumor was excised and weighed. A compound is considered active in this system if it produces a tumor weight regression >58% (T/C ≤42%) compared to controls.

In the present study, a computer search was made of the NCI biological database for active 2-, 6-, and 8-substituted purines. 8-Substituted purines do not appear in the final correlation because there were too few active compounds to warrant their inclusion. Compounds containing SH or OH groups were excluded because these drugs exist as tautomers and no true substituent constants are available. Admittedly, this may narrow the conclusions somewhat. It has been shown, however, that one of the active forms of mercaptopurine and thioguanosine is the 6-methylthio derivative of each of those compounds obtained by *in vivo S*-methylation (12), and these analogs have been included in this study. Aminopurines, which have been shown to exist mainly in the NH_2 form (13), were included. Dose response plots were generated and the standard biological response was taken at T/C = 20%. C is the dose (moles/kg) that produces this response.

The toxicity correlation was restricted to 6-monosubstituted purines, since these comprise the major portion of purine bases tested by the NCI. The biological database was searched for those compounds for which a dose–response curve could be constructed and which showed reproducible toxicity. The LD_{50} (Table III), in moles/kg, was calculated by the probit method (14) from toxicity day (Day 11) survivors (15).

RESULTS AND DISCUSSION

Equation 1 was generated from the data in Tables I and II. The development of Eq. 1 is given in Table IV.

Table III—Observed and Predicted Toxicities of 6-Substituted Purines

Number	Substituent	log(1/LD_{50}) Observed	log(1/LD_{50}) Predicted[a]	|Δlog(1/LD_{50})|
I	Purine	Nontoxic	2.91	—
II	Chloro	2.73	2.91	0.18
III	Bromo	2.79	2.94	0.15
IV	Iodo	2.96	3.00	0.04
IX	Methylthio	3.16	3.07	0.09
X	Ethylthio	3.04	3.09	0.05
XXIX	Cyano	3.26	3.10	0.16
XXX	Ethoxy	3.24	3.08	0.16
XXXI	Amino	3.10	3.18	0.08
XXXII	Hydrazino	3.24	3.19	0.05
XXXIII	*n*-Propylthio	3.21	3.12	0.09
XXIV	*n*-Butylthio	3.06	3.14	0.08
XXXV	iso-Butylthio	3.15	3.12	0.03
XXXVI	*sec*-Butylthio	3.02	3.12	0.10
XXXVII	2′-Propynylthio	3.12	3.16	0.04
XXXVIII	Cyanomethylthio	3.35	3.29	0.06
XXXIX	Cyclopentylthio	3.23	3.14	0.09
XL	Phenylthio	3.32	3.22	0.10
XLI	2′-Imidazolinylthio	3.38	3.33	0.05
XLII	1′-Methyl-4′-nitro-imidazol-5′-ylthio[b]	3.60	3.55	0.05
XLIII	Benzylthio	3.13	3.25	0.12
XLIV	3′,4′-Dimethyl-benzylthio	3.20	3.28	0.08
LXV	*o*-Chlorobenzylthio	3.04	3.05	0.01
XLVI	*o*-Fluorobenzylthio	3.31	3.19	0.12
XLVII	*o*-Nitrobenzylthio	3.42	3.41	0.01
LXVIII	*m*-Chlorobenzylthio	3.22	3.23	0.01
L	*p*-Fluorobenzylthio	3.28	3.19	0.09
LI	2′-Pyridylmethylthio	3.38	3.43	0.05
LII	Ethylsulfonyl	3.30	3.41	0.11
LII	*n*-Propylsulfonyl	3.39	3.43	0.04
LIV	*n*-Butylsulfonyl	3.43	3.44	0.01
LV	*N*-Methylsulfon-amido	3.53	3.42	0.11
LVI	*N*-*n*-Propylsulfon-amido	3.43	3.45	0.02
LVII	*N*-iso-Butylsulfon-amido	3.58	3.50	0.08
LVIII	*N*-3′-Methoxypropyl-sulfonamido	3.48	3.58	0.10
LIX	*N*-2′-Ethoxyethyl-sulfonamido	3.57	3.59	0.02
LX	*N*-Benzylsulfonamido	3.58	3.65	0.07

[a] From Eq. 3. [b] Azathioprine.

$$\log(1/C) = 4.26(\pm 0.24) - 0.47(\pm 0.37)\ \text{MR}(2) + 1.18(\pm 0.55)\ \mathcal{R} \quad (6)$$

$$n = 22;\ r = 0.815;\ s = 0.372 \quad \text{(Eq. 1)}$$

Equation 1 relates the CA755 antitumor potency of 2,6-mono- and disubstituted purines to the molar refractivity of substituents at position 2 and the resonance constant $\mathcal{R}$ at position 6. The negative coefficient of the MR (2) term indicates that bulky substituents at position 2 are to be avoided for maximum potency in this tumor system. The larger positive coefficient of the $\mathcal{R}$ term suggests that potency in CA755 is predominantly influenced by the resonance effect of groups at position 6. Strong electron-withdrawing groups would be expected to increase potency. This is consistent with the observation that the 6-methylthio derivative ($\mathcal{R} = -0.11$) routinely produced complete tumor regression (T/C = 0%) at doses of 20 mg/kg. The 6-methoxy derivative ($\mathcal{R} = -0.51$), by comparison, requires ~10 times as much drug (250 mg/kg) to produce the same effect.

The 6-halo analogs (6-Cl, 6-Br, and 6-I) have about the same $\mathcal{R}$ values (−0.15, −0.17, and −0.19) as the 6-methylthio derivative and exhibit about the same potency as the latter. The 6-thiocyanate derivative ($\mathcal{R}$ = +0.19) was an outlier and was not included in Eq. 1. It was, however, one of the most potent compounds tested by the NCI, producing complete tumor regression at ~7 mg/kg. This analog may be, as suggested previously (16), a masked form of mercaptopurine, which may be produced by *in vivo* reduction.

The correlation coefficient of Eq. 1 is quite reasonable, considering the inherent variability in tumor weigh measurements. It accounts for ~66% of the variance in the biological data ($r^2 = 0.664$).

Equation 2, a toxicity correlation, was derived from the data in Tables I and III and includes only 6-thiopurines:

Table IV—Development of Equation 1

Intercept	MR (2)	$\mathcal{R}$ (6)	r	s	$F_{1,x}$	p	Equation
4.03	−0.72	—	0.560	0.519	$F_{1,21}$ = 9.16	<0.01	A
4.13	—	1.39	0.736	0.424	$F_{1,21}$ = 23.63	<0.001	B
4.26	−0.47	1.18	0.815	0.372	$F_{1,20}$ = 6.92[a]	<0.025	C

[a] This F value was obtained in comparison with Eq. B.

Table V—Development of Equation 3

Intercept	π(6)	MR (6)	r	s	$F_{1,x}$	p	Equation
3.30	−0.05	—	0.342	0.202	$F_{1,35}$ = 4.49	<0.05	A
3.04	—	0.08	0.452	0.192	$F_{1,35}$ = 8.73	<0.01	B
2.89	−0.15	0.19	0.908	0.092	$F_{1,34}$ = 116.06[a]	<0.001	C

[a] This F value of Eq. C is obtained in comparison with Eq. B.

$$\log(1/LD_{50}) = 2.95(\pm0.12) - 0.14(\pm0.02)\pi\ (6) + 0.17(\pm0.04)\text{MR}\ (6)$$
$$n = 29;\ r = 0.911;\ s = 0.076 \qquad \text{(Eq. 2)}$$

Equation 2 indicates that toxicity of 6-substituted thiopurines is influenced to about the same extent by the lipophilicity and bulkiness of groups at position 6. Toxicity would be expected to decrease with increasing lipophilicity. Bulky groups would be expected to increase the toxicity. To ensure that this was not a phenomenon peculiar to 6-thiopurines, seven additional compounds without sulfur bonds (II–IV, XXIX–XXXII) were included in the following:

$$\log(1/LD_{50}) = 2.89(\pm0.08) - 0.15(\pm0.03)\pi\ (6) + 0.19(\pm0.03)\ \text{MR}\ (6)$$
$$n = 36;\ r = 0.908;\ s = 0.092 \qquad \text{(Eq. 3)}$$

The development of Eq. 3 is given in Table V. Equation 3 is identical to Eq. 2 and appears to express a general structure–activity relationship for 6-substituted purines. In Eq. 3, the positive effect of increasing π (6) in order to decrease toxicity is offset by the toxicity-increasing effect of MR (6). This phenomenon has been observed in other systems[1], but the biochemical implications are not at all clear. There is some intercorrelation between MR (6) and π (6) (arc cos 0.38 = 68°) (17) as might be expected.

Equation 3 reflects the rather limited range of toxicities of 6-monosubstituted purines (Table III). Viewed together with Eq. 3, Eq. 1 suggests that, other things being equal, the development of more potent 6-substituted purines is not likely to result in a substantial increase in toxicity.

Few cancer QSAR studies have been published to date (18). This may be due both to the complexity of the problem and the nature of the data. Nevertheless, those studies that have been published show that cancer data will yield reasonable structure–activity correlations (15, 19, 20). In the present study, an attempt has been made to employ tumor weight inhibition data and standard physicochemical parameters in order to derive QSAR for an important class of antitumor agents. The correlations presented are reasonable and consistent with the data. The results suggest that other biological data obtained from the study of purines (enzyme inhibition data, *in vivo* survival data, *etc.*) may yield satisfactory correlations as well.

[1] Personal communication, C. Hansch, Seaver Chemistry Laboratory, Pomona College, Claremont, CA 91711.

REFERENCES

(1) J. A. Montgomery, in "Antineoplastic and Immunosuppressive Agents I," A. C. Sartorelli and D. G. Johns, Eds., Springer-Verlag, Berlin, 1974, chap. 5.

(2) J. H. Lister, "Purines," Wiley-Interscience, New York, N.Y., 1971, p. 9.

(3) W. H. Cone, in "Chemotherapy of Cancer," Lea & Febiger, Philadelphia, Pa., 1970, chap. 1.

(4) Z. Neiman and F. R. Quinn, *J. Pharm. Sci.*, **70,** 425 (1981).

(5) Z. Neiman, *Experientia*, **31,** 996 (1975).

(6) H. E. Skipper, J. A. Montgomery, J. R. Thompson, and F. M. Schabel, Jr., *Cancer Res.*, **19,** 425 (1958).

(7) R. I. Geran, N. H. Greenberg, M. M. MacDonald, A. M. Schumacher, and B. J. Abbott, *Cancer Chemother. Rep., Part 3*, **3,** 1 (1972).

(8) C. Hansch, A. Leo, S. H. Unger, K. H. Kim, D. Nikaitani, and E. J. Lien, *J. Med. Chem.*, **16,** 1207 (1973).

(9) C. Hansch and A. Leo, "Substituent Constants for Correlation Analysis in Chemistry and Biology," Wiley, New York, N.Y., 1979, Appendix I.

(10) *Ibid.*, Appendix II.

(11) A. I. Vogel, "A Text-Book of Practical Organic Chemistry," 3rd ed., Longman, London, 1970, p. 1036.

(12) L. S. Goodman and A. Gilman, in "The Pharmacological Basis of Therapeutics," 5th ed., Macmillan, New York, N.Y., 1975, p. 1279.

(13) J. H. Lister, "Purines," Wiley-Interscience, New York, N.Y., 1971, p. 8.

(14) A. J. Barr, J. H. Goodnight, J. P. Sall, and J. T. Helwig, "Statistical Analysis System," SAS Institute, Cary, N.C., 1979.

(15) F. R. Quinn, Z. Neiman, and J. Beisler, *J. Med. Chem.*, **24,** 636 (1981).

(16) T. Nagamachi, J. Fourrey, P. F. Torrence, J. A. Waters, and B. Witkop, *ibid.*, **17,** 403 (1974).

(17) S. H. Unger and C. Hansch, *ibid.*, **16,** 745 (1973).

(18) C. Hansch, *Farmaco, Ed. Sci.*, **34,** 89 (1979).

(19) C. Hansch, in "Correlation Analysis in Chemistry," N. B. Chapman and J. Shorter, Eds., Plenum, New York, N.Y., 1978, p. 426.

(20) F. R. Quinn and J. A. Beisler, *J. Med. Chem.*, **24,** 251 (1981).