Structure–Activity Relationships of Benzohydroxamic Acid Inhibitors of Ribonucleotide Reductase

BART van't RIET *x, LEMONT B. KIER *, and HOWARD L. ELFORD ‡

Received July 5, 1979, from the **Department of Pharmaceutical Chemistry and the* ‡*Department of Biochemistry, Medical College of Virginia, Virginia Commonwealth University, Richmond, VA 23298.* Accepted for publication January 31, 1980.

Abstract □ A structure–activity relationship study of 28 substituted benzohydroxamic acids that inhibit ribonucleotide reductase was undertaken to discern the structural features of the molecule contributing to the inhibitory potency of these compounds. An equation containing three molecular connectivity indexes, but not including Hammett σ values, was developed which gives close correlation with observed values for ribonucleotide reductase inhibition. It is postulated that the inhibitory potency involves two parts of the benzohydroxamic acid molecule. One is the hydroxamic portion, which complexes with the metal component of the enzyme, providing a qualitative effect. The other is an interaction involving the benzene ring and its substituents and may provide the quantitative aspect of the observed inhibition values.

Keyphrases □ Hydroxyurea, analogs—substituted aryl hydroxamic acids, inhibition of ribonucleotide reductase, structure–activity relationships, molecular connectivity indexes □ Structure–activity relationships—substituted aryl hydroxamic acids, inhibition of ribonucleotide reductase, molecular connectivity indexes □ Molecular connectivity indexes—substituted aryl hydroxamic acids, inhibition of ribonucleotide reductase, structure–activity relationships □ Antitumor agents—substituted aryl hydroxamic acids, inhibition of ribonucleotide reductase, structure–activity relationships

The reduction of ribonucleotides to deoxyribonucleotides is a rate-limiting step in DNA biosynthesis and, hence, cellular replication. The required enzyme, ribonucleotide reductase, is inhibited by hydroxyurea, which presently is the only clinical antitumor agent with this primary mode of action. Replacement of the amino group of hydroxyurea by various aryl groups gives hydroxamic acids that inhibit ribonucleotide reductase at concentrations lower than those of hydroxyurea (1). The aryl hydroxamic acids also prolong the life of mice with L1210 leukemia (2, 3). Several aryl hydroxamic acids were reported to inhibit DNA synthesis (4, 5).

The possibility of improving on existing chemotherapeutic agents led to an examination of the relationship between the enzyme inhibitory potency and the structures of 28 pyridyl and benzohydroxamic acids, which were tested in this laboratory.

METHODS

Molecular structures can be analyzed in terms of the number of atoms, the kind of atoms, bonding types, and the adjacent environment by the molecular connectivity method (6–10). Molecular connectivity indexes have been computed for each compound in this study (7). In addition, the Hammett σ values for the substituents were summed for each compound and included as an electronic contribution variable. A multivariable search for the best correlation was conducted in a regression analysis.

The synthesis and testing of the *in vitro* inhibitory potency of the aryl hydroxamic acids were reported elsewhere (1, 3). The concentration for the ID_{50} used in this study is expressed as the pC value (Table I). The pC values were determined graphically from at least three concentrations within 1 log unit after determining the inhibition range. In repetitive experiments, the observed pC values agreed within 0.15 pC unit.

RESULTS AND DISCUSSION

A search for the best three-variable equation relating potency (expressed as pC) and structure (encoded with connectivity indexes) was conducted. Hammett σ values were included in the search but did not appear in any equation with a high correlation. The best equation is:

$$pC = 2.36(\pm0.04)^3\chi_p - 3.98(\pm0.53)^0\chi^v + 0.97(\pm0.05)(^1\chi^v)^2 + 9.20(\pm5.50) \quad \text{(Eq. 1)}$$

$$r = 0.943 \quad s = 0.21 \quad n = 28 \quad F = 64$$

Equation 1 explains about 90% of the variation in the data. Because of the experimental variation in pC determinations, the equation is nearly as good as can be expected from the data. Predicted values are found in Table I.

The $^3\chi_p$ index is a weighted count of contiguous three-path fragments, the $^0\chi^v$ index is based on atom deltas, and the $^1\chi^v$ index is a weighted count of bonds. The last two indexes are based on valence deltas. The connectivity index is computed by the general formula $^m\chi_t = \Sigma(\delta_i\delta_j \ldots \delta_n)^{-1/2}$, where m is the order (number of bonds), t is the type (the path in these cases), and the number of deltas in parentheses is equal to the number of bonded atoms in the fragment of order m.

It is apparent that the $^3\chi_p$ index quantifies a structure fragment that plays a favorable role in the pC value. The $^3\chi_p$ index can discriminate among compounds having the same number of substituents in different substitution patterns. Thus, the $^3\chi_p$ index is derived from an additional subgraph wherever two ring substituents are *ortho* to each other. Equation 1 thus describes this structural condition as more favorable for inhibitory potency than the alternative cases.

This situation is illustrated in several examples. Compounds IV, VI, and VIII have *ortho*-, *meta*-, and *para*-hydroxy substitution, respectively, and have the corresponding pC values of 3.82, 3.46, and 3.60. An identical case is found in the comparison of the monoamino derivatives, V, VII, and IX. Again, the *ortho*-substituent is described correctly by the equation as having a larger $^3\chi_p$ term and, hence, a larger pC value. Disubstituted derivatives reinforce this structural analysis (compare XIII–XVIII). Finally, XXVI is correctly described as having the largest pC value by virtue of having four adjacent ring substituents.

The influence of the $^0\chi^v$ and $^1\chi^v$ indexes essentially offsets the information encoded in $^3\chi_p$ (i.e., increasing size increases potency). This result leaves the $^3\chi_p$ index with the exclusive role of encoding the favorable influence of *ortho*-substituents.

The failure to find the Hammett σ term in any high correlation equation indicates that the electronic contribution of the ring substituents is not a prominent factor in the inhibitory potency.

Further evidence for this conclusion can be found in the direct comparison of several compounds. Compounds IV, V, and IX have significantly different Hammett σ values, but their pC values are close. Compounds VI and VII have appreciably different σ values whereas their observed pC values are the same. Thus, regression analysis reveals that electronic effects of the substituents are not prominent but that structural features encoded in the molecular connectivity indexes can account for most of the systematic variation in the pC values in this set of compounds.

These observations indicate that the ring substituents play a role in the inhibitory potency of the hydroxamic acid molecule. This role could take the form of an interaction involving the ring and its substituents or an interaction of the substituents themselves with the active site. Thus, the lack of an electronic contribution to the ring indicates that the general region of the ring must interact with an enzyme binding site. Therefore, it is postulated that two types of interaction occur: (*a*) an underlying constant activity of the hydroxamic acid function with the metal component of the enzyme and (*b*) an additional interaction due to the ring

0022-3549/80/0700-0856$01.00/0

Table I—Calculated Inhibitory Potencies of Aryl Hydroxamic Acids

RCNHOH
‖
O

Compound	R	$^3\chi_p$	$^0\chi^v$	$^1\chi^v$	pC Found	pC Calc.
I	Pyridyl-2	3.099	5.112	2.698	3.30	3.23
II	Pyridyl-3	3.099	5.112	2.688	3.10	3.17
III	Unsubstituted phenyl	3.099	5.242	2.838	3.40	3.46
IV	2-Hydroxyphenyl	3.553	5.612	2.979	3.82	3.85
V	2-Aminophenyl	3.553	5.742	3.044	3.92	3.71
VI	3-Hydroxyphenyl	3.426	5.612	2.973	3.46	3.52
VII	3-Aminophenyl	3.426	5.742	3.038	3.46	3.38
VIII	4-Hydroxyphenyl	3.509	5.612	2.972	3.60	3.71
IX	4-Aminophenyl	3.509	5.742	3.038	3.82	3.57
X	4-Methylaminophenyl	3.917	6.665	3.499	3.48	3.79
XI	4-Dimethylaminophenyl	4.208	7.612	3.867	3.30	3.33
XII	4-Methoxyphenyl	3.917	6.573	3.361	3.30	3.24
XIII	2,3-Dihydroxyphenyl	4.145	5.982	3.119	5.10	4.60
XIV	2,4-Dihydroxyphenyl	3.873	5.982	3.113	3.60	3.92
XV	2,5-Dihydroxyphenyl	3.895	5.982	3.113	3.70	3.98
XVI	2,6-Dihydroxyphenyl	3.934	5.982	3.119	4.00	4.11
XVII	3,4-Dihydroxyphenyl	4.087	5.982	3.113	4.52	4.43
XVIII	3,5-Dihydroxyphenyl	3.664	5.982	3.107	3.40	3.40
XIX	2-Hydroxy-3-methylphenyl	4.145	6.535	3.395	3.82	4.15
XX	2-Hydroxy-4-aminophenyl	3.873	6.112	3.178	3.70	3.80
XXI	3,4-Dimethylphenyl	4.087	7.088	3.666	3.52	3.67
XXII	3,4-Diaminophenyl	4.087	6.242	3.243	4.40	4.20
XXIII	3,4-Dimethoxyphenyl	4.678	7.904	3.891	3.60	3.46
XXIV	2,4-Dichlorophenyl	3.873	7.478	3.861	3.35	3.03
XXV	3,4-Dichlorophenyl	4.087	7.478	3.861	3.60	3.54
XXVI	2,3,4-Trihydroxyphenyl	4.732	6.352	3.259	5.46	5.38
XXVII	3,4,5-Trihydroxyphenyl	4.593	6.352	3.253	5.00	5.02
XXVIII	3,4,5-Trimethoxyphenyl	5.391	9.235	4.420	4.00	4.10

and its substituents to give the quantitative differences found in enzyme inhibition values.

REFERENCES

(1) B. van't Riet, G. L. Wampler, and H. L. Elford, *J. Med. Chem.*, **22**, 589 (1979).

(2) H. L. Elford, *Proc. Am. Assoc. Cancer Res.*, **18**, 177 (1977).

(3) H. L. Elford, G. L. Wampler, and B. van't Riet, *Cancer Res.*, **39**, 844 (1979).

(4) G. R. Gale and J. B. Hynes, *J. Med. Chem.*, **11**, 191 (1968).

(5) G. R. Gale, J. B. Hynes, and A. B. Smith, *ibid.*, **13**, 571 (1970).

(6) L. B. Kier, L. H. Hall, W. J. Murray, and M. J. Randic, *J. Pharm. Sci.*, **64**, 1971 (1975).

(7) L. B. Kier and L. H. Hall, "Molecular Connectivity in Chemistry and Drug Research," Academic, New York, N.Y., 1976.

(8) L. B. Kier and R. A. Glennon, *Life Sci.*, **22**, 1589 (1978).

(9) T. DiPaolo, L. B. Kier, and L. H. Hall, *Mol. Pharmacol.*, **13**, 31 (1977).

(10) G. R. Parker, *J. Pharm. Sci.*, **67**, 513 (1978).

ACKNOWLEDGMENTS

Supported by American Cancer Society Grant IN-105C and National Cancer Institute Grant CA 21305.

The authors thank Roberta Elford for editorial assistance.

Sensitive Method for Determination of Ethinyl Estradiol in Presence of Norethindrone

SHINICHIRO HIRAI, ANWAR HUSSAIN ˣ, and SALIM BABHAIR

Received November 14, 1979, from the *College of Pharmacy, University of Kentucky, Lexington, KY 40506.* Accepted for publication February 8, 1980.

Abstract □ A sensitive spectrophotofluorometric procedure for the determination of microamounts of ethinyl estradiol is described. The method is useful for the determination of ethinyl estradiol in the presence of norethindrone and common tablet excipients, especially in dissolution media.

Keyphrases □ Ethinyl estradiol—spectrophotofluorometric analysis in the presence of norethindrone and common tablet excipients □ Spectrophotofluorometry—analysis, ethinyl estradiol in the presence of norethindrone and common tablet excipients □ Dissolution rates—ethinyl estradiol in tablets, spectrophotofluorometric analysis in the presence of norethindrone and common tablet excipients

To study the dissolution rate of ethinyl estradiol and norethindrone from tablets, a sensitive procedure is needed to determine microamounts of these drugs in the presence of each other. The spectrophotometric methods described in the USP (1) for the determination of the two drugs require at least 20 tablets of each and, consequently, cannot be used to determine very small amounts of ethinyl estradiol in the dissolution medium, especially in the pres-

0022-3549/80/0700-0857$01.00/0