Biochemical Pharmacology, Vol. 23, pp. 2421–2429. Pergamon Press, 1974. Printed in Great Britain.

COMPARATIVE STUDIES OF THE ANTINEOPLASTIC ACTIVITY OF 5-HYDROXY-2-FORMYLPYRIDINE THIOSEMICARBAZONE AND ITS SELENO-SEMICARBAZONE, GUANYLHYDRAZONE AND SEMICARBAZONE ANALOGS*

KRISHNA C. AGRAWAL, BARBARA A. BOOTH, RICHARD L. MICHAUD,† E. COLLEEN MOORE and ALAN C. SARTORELLI

Department of Pharmacology, Yale University School of Medicine, New Haven, Conn. 06510, and Department of Biochemistry, The University of Texas Cancer Center, M. D. Anderson Hospital and Tumor Institute at Houston, Houston, Tex. 77025, U.S.A.

(*Received* 11 *November* 1973; *accepted* 1 *February* 1974)

Abstract—Several analogs of 5-hydroxy-2-formylpyridine thiosemicarbazone (5-HP) that contain isosteric replacement of sulfur by Se, NH or O have been synthesized. Measurement of the antineoplastic activity of these compounds in mice bearing Sarcoma 180 ascites cells indicated that 5-HP was the most active of these agents; the seleno analog was intermediate in potency, and the guanylhydrazone and semicarbazone were inactive against this tumor. 5-HP and its seleno analog both caused marked inhibition of DNA synthesis *in vitro*, as measured by the incorporation of thymidine-methyl-^{3}H, 5-^{3}H-cytidine or adenine-8-^{14}C into DNA. The syntheses of RNA and protein were relatively unaffected by these agents under the conditions employed. None of the compounds prevented the incorporation of 5-^{3}H-cytidine into acid-soluble pyrimidine ribonucleotides, but 5-HP and its selenosemicarbazone markedly depressed the subsequent progression of radioactivity into pyrimidine deoxyribonucleotide pools, suggesting that these two derivatives inhibited the enzyme ribonucleoside diphosphate reductase *in situ*. Both 5-HP and the seleno analog inhibited the isolated enzyme ribonucleoside diphosphate reductase from the Novikoff rat hepatoma; to achieve 50 per cent inhibition required $3{\cdot}5 \times 10^{-6}$ and $6{\cdot}8 \times 10^{-6}$ M respectively. Metal/ligand ratios and the association constants for these ligands with cobalt and copper were determined. The cobalt/ligand ratio for 5-HP and its seleno analog was 1:2 and the copper/ligand ratio for these agents was 1:1. Metal/ligand ratios for the guanylhydrazone and semicarbazone derivatives were 1:3 for cobalt and 1:2 for copper. Association constants for 5-HP and the seleno derivative with cobalt were 56×10^{9} and $7{\cdot}6 \times 10^{9}$ respectively. The findings demonstrated that 5-HP was the optimum member of this series with respect to antineoplastic potency and that tumor-inhibitory activity correlated with the degree of inhibition of the synthesis of DNA and the capacity for metal binding.

5-HYDROXY-2-FORMYLPYRIDINE thiosemicarbazone (5-HP) has been under active investigation as an anticancer drug, both in experimental animal systems and in humans.[1–8] 5-HP is a member of a class of α-(*N*)-heterocyclic carboxaldehyde thiosemicarbazones that has demonstrated pronounced growth-inhibitory activity against a broad spectrum of transplanted rodent neoplasms,[9] spontaneous lymphomas of dogs,[5] and DNA viruses of the Herpes group.[10] Members of this drug class generally

* This research was supported in part by Grants CA-02817 and CA-04464 from the National Cancer Institute, USPHS.

† Present Address: St. John's College, Santa Fe, New Mexico 87501.

have poor water solubility, which limits their potential usage in man by parenteral administration;[2] 5-HP was selected as the initial candidate for clinical trials because: (a) it is an active antineoplastic agent against rodent transplanted tumors, (b) its therapeutic index is relatively great compared to other members of the series, and (c) it could be solubilized in water as the sodium salt of the phenolic hydroxyl group. Although weak antileukemic activity has been attained in patients by the administration of 5-HP, a serious disadvantage was severe gastrointestinal toxicity manifested by nausea and vomiting.[7,8]

It has been shown by our laboratory that 5-HP inhibits primarily the synthesis of DNA.[11] The site of the metabolic lesion on the DNA biosynthetic pathways is the enzyme ribonucleoside diphosphate reductase (EC 1.17.4.1), which is responsible for the generation of the deoxyribonucleotide precursors of DNA.[12] Kinetic studies on the molecular mechanism of action of this class of compounds have implicated the interaction of 5-HP as a tridentate ligand (N*-N*-S*) with iron required for the expression of ribonucleoside diphosphate reductase activity.[13] To assess the relative importance of metal-binding to the action of 5-HP (i.e. inhibition of DNA synthesis and of tumor growth), several analogs of 5-HP have been synthesized by isosteric replacement of sulfur in the side chain with Se, NH or O.

MATERIALS AND METHODS

Chemical methods

Melting points of the compounds were determined using a Thomas–Hoover capillary melting point apparatus and are uncorrected. Elemental analyses were performed by the Schwarzkopf Microanalytical Laboratory, Woodside, N.Y. Ultraviolet spectra were carried out using a Zeiss spectrophotometer model PMQ II.

5-Hydroxy-2-formylpyridine, the common intermediate for the synthesis of various analogs, was prepared by published procedures.[1] The various reactions employed for the preparation of isosteric analogs are shown in Fig. 1. Since selenosemicarbazide has been reported to be unstable,[14] synthesis of the seleno derivative was accomplished by utilizing a displacement reaction. First, acetonyl selenosemicarbazone was

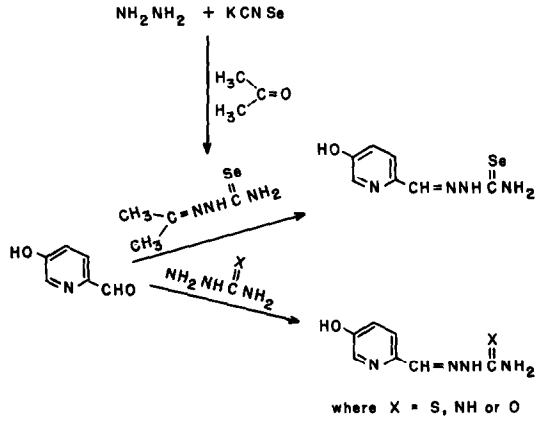

FIG. 1. Reactions employed for the preparation of isosteric analogs of 5-HP.

formed by reacting potassium selenocyanate with hydrazine in the presence of acetone according to the procedure of Huls and Renson.[15] Treatment of acetonylselenosemicarbazone with 5-hydroxypyridine-2-carboxaldehyde resulted in the desired compound. Thiosemicarbazone, guanylhydrazone and semicarbazone derivatives were synthesized by reacting the aldehyde with thiosemicarbazide, aminoguanidine or semicarbazide respectively.

5-Hydroxy-2-formylpyridine selenosemicarbazone. Acetonyl selenosemicarbazone[15] (0·178 g, 1 m-mole) was dissolved in 20 ml hot water and a solution of 5-hydroxy-2-formylpyridine (0·123 g, 1 m-mole) in 10 ml water was added. The resulting solution was heated for 5 min after addition of 2 drops of dilute acetic acid. The solution was then concentrated to one-half the volume under vacuum and cooled. Selenosemicarbazone was filtered, washed with water and recrystallized from ethanol and water to yield 0·12 g (50%), m.p., 214–215° dec.

Anal. Calcd. for $C_7H_8N_4OSe$: C, 34·57; H, 3·29; N, 23·05. Found: C, 34·36; H, 3·30; N, 22·92.

5-Hydroxy-2-formylpyridine guanylhydrazone. A solution of aminoguanidine bicarbonate (0·272 g, 2 m-moles) in 5 ml of dilute acetic acid was mixed with a solution of 5-hydroxy-2-formylpyridine (0·246 g, 2 m-moles) in 10 ml water. The mixture was heated for 10 min and evaporated to dryness under vacuum. The residue was washed with a small amount of ethanol and redissolved in 5 ml water, neutralized with sodium acetate and allowed to crystallize overnight in the refrigerator. The guanylhydrazone was filtered, washed with cold water and dried. Recrystallization from ethanol and ethyl acetate yielded 0·14 g (40%), m.p., 210–212° dec.

Anal. Calcd. for $C_7H_9N_5O$: C, 46·93; H, 5·03; N, 39·11. Found: C, 47·12; H, 5·25; N, 38·83.

5-Hydroxy-2-formylpyridine semicarbazone. 5-Hydroxy-2-formylpyridine (0·246 g, 2 m-moles) was added to a solution of semicarbazide hydrochloride (0·223 g, 2 m-moles) in 5 ml water and heated for 10 min. The resulting solution was neutralized with sodium acetate and allowed to crystallize. The semicarbazone was filtered and recrystallized from ethanol and water to yield 0·22 g (60%), m.p., 217–218° dec.

Anal. Calcd. for $C_7H_8N_4O_2$: C, 46·67; H, 4·44; N, 31·11. Found: C, 46·43; H, 4·49; N, 30·92.

Metal affinity. α-(*N*)-Heterocyclic carboxaldehyde thiosemicarbazones have been reported to form coordination compounds with ferrous ions.[16] The ability of these compounds to coordinate ferrous ions was paralleled by their capacities to bind cobalt. Because of difficulties encountered with ferrous ions due to oxidation, cobalt and copper were used as model systems in this study. The ligand/metal binding ratios and the association constants were determined for both metals. This was accomplished by Job's method of continuous variation,[17] using spectrophotometric absorbance shifts in the near u.v. and visible range as indices of complex formation. The studies were carried out in methanol.

Biological methods

Compounds were tested for antineoplastic activity in mice bearing Sarcoma 180 ascites cells; complete details of assays for tumor inhibition have been described earlier.[18] Transplantation of the neoplasm was accomplished by inoculating mice intraperitoneally with approximately 6×10^6 ascites cells. Drugs were administered by

intraperitoneal injection beginning 24 hr after tumor implantation and treatment was continued once daily for 6 consecutive days. Determination of the sensitivity of Sarcoma 180 to these agents was based upon the prolongation of survival time afforded by the drug treatments.

To evaluate the direct effects of these agents on the neoplastic cells present in the peritoneal cavities of mice, single doses of drugs (20 mg/kg) were injected intraperitoneally on the fifth day after tumor transplantation. Twenty-four hr later, ascites cells were quantitatively washed from the peritoneal cavity with saline, the suspension was treated with 0·5% trypsin to reduce clumping, and the cells were counted with a Coulter model A particle counter. Measurement of the average cell volume was done in hematocrit tubes as previously described.[11]

Biochemical studies

Ribonucleoside diphosphate reductase was partially purified from rat Novikoff ascites tumor cells as previously reported.[19] Reduction of CDP-^{32}P (0·17 mM, 1–2 × 10^6 cpm/μmole) was assayed in 8·3 mM potassium phosphate buffer, pH 7·0, using 2·1 mM ATP as allosteric activator, 6·3 mM dithioerythritol or dithiothreitol as the dithiol substrate, and 42 μM $(NH_4)_2Fe(SO_4)_2$ and 4·2 mM $Mg(C_2H_3O_2)_2$ as the metal cofactors. Approximately 0·15 mg enzyme protein was used per 0·12 ml of incubation mixture. The enzyme was added to an ice-cold mixture of substrates and inhibitors, immediately warmed to 37° and incubated 30 min. The reaction was terminated by addition of 1 ml of 1M perchloric acid (PCA).

Studies *in vitro* were carried out in duplicate on pooled 6-day growths of Sarcoma 180 ascites cells. Approximately 1·5 × 10^8 cells were incubated for 30 min at 37° in a total volume of 10 ml of Robinson's medium minus calcium chloride, pH 7·4,[20] with or without inhibitors which were dissolved in dimethylsulfoxide (DMSO) and diluted to yield a final concentration of DMSO no greater than 4 per cent. Each experiment was controlled by duplicate flasks containing comparable levels of DMSO instead of drug. Cytidine-5-^{3}H (40μg/flask; 4·5 × 10^4 cpm/μg), adenine-8-^{14}C (40 μg/flask; 4·5 × 10^4 cpm/μg) and thymidine-methyl-^{3}H (40 μg/flask; 8·0 × 10^4 cpm/μg) were utilized to monitor nucleic acid biosynthetic pathways. D,L-Leucine-l-^{14}C (400 μg/flask; 5·3 × 10^4 cpm/μg) was used to measure the rate of protein synthesis. Cytidine-5-^{3}H incorporation into the acid-soluble fraction was analyzed according to procedures described earlier.[12] Analysis of experiments with thymidine-methyl-^{3}H, adenine-8-^{14}C and cytidine-5-^{3}H as precursors of nucleic acid synthesis were conducted as previously reported.[21]

Radioactivity was measured in a Packard Tri-Carb liquid scintillation spectrometer using as the phosphor solution Liquifluor (New England Nuclear Corp.).

RESULTS AND DISCUSSION

The effects of 5-HP and its analogs on the survival time of mice bearing Sarcoma 180 ascites cells are shown in Table 1. Administration of 5-HP at its maximum effective daily dosage level of 40 mg/kg caused an extension of lifespan of tumor-bearing animals from 13·8 days for untreated controls to 25·8 days. Larger dose levels of this agent were found to cause toxicity, as shown by a 10·2 per cent loss in host body weight. Selenosemicarbazone at its maximum effective daily dose of 60 mg/kg was

TABLE 1. EFFECT OF 5-HYDROXY-2-FORMYLPYRIDINE THIOSEMICARBAZONE (5-HP) AND ITS ANALOGS ON THE SURVIVAL TIME OF MICE BEARING SARCOMA 180 ASCITES CELLS

Derivative of 5-hydroxy-2-formylpyridine	Daily dosage* (mg/kg)	Average Δwt† (%)	Average survival (days ± S.E.)
None		+15·0	13·8 ± 0·5
Thiosemicarbazone (5-HP)	20	+ 3·8	21·0 ± 3·1
	40	+ 2·0	25·8 ± 6·2 (1/5)‡
	60	−10·2	23·9 ± 3·5 (1/10)‡
Selenosemicarbazone	20	+12·2	16·7 ± 2·3
	40	+ 5·0	15·9 ± 0·6
	60	− 1·8	19·8 ± 2·4
Guanylhydrazone	20	+ 9·5	13·0 ± 0·7
	40	+15·6	12·7 ± 0·7
	60	+ 6·7	9·6 ± 1·2
Semicarbazone	20	+13·8	13·7 ± 0·5
	40	+10·4	11·6 ± 1·2
	60	+14·8	10·9 ± 0·7

* Administered once daily for 6 consecutive days, beginning 24 hr after tumor implantation.

† Average weight change from onset to termination of drug treatment.

‡ Number of 50-day survivors. Mice surviving over 50 days were calculated as 50-day survivors in the determination of the average survival time. Each value represents the results obtained using 5–10 mice.

less effective than 5-HP on a molar basis, increasing the average survival time of tumor-bearing mice by only 6 days. The other two analogs, the guanylhydrazone and the semicarbazone, were ineffective as tumor inhibitors at the dosage levels employed. These agents also did not appear to cause toxicity, as measured by loss in body weight, but the largest doses were found to decrease the average survival time.

Since these results are an expression of the relative toxicities of these agents for both host and tumor tissues and, for this reason, precise comparisons with biochemical studies cannot be made, the direct effects of the compounds on Sarcoma 180 ascites cells in the peritoneal cavity under conditions identical to those used for some

TABLE 2. TOXICITY OF 5-HYDROXY-2-FORMYLPYRIDINE THIOSEMICARBAZONE (5-HP) AND ITS ANALOGS TO SARCOMA 180 ASCITES CELLS*

Derivative of 5-hydroxy-2-formylpyridine	Total cells/mouse $\times 10^{-6}$	Average cell volume (μ^3/cell $\times 10^{-3}$)
None (zero time)	262 ± 21	4·2 ± 0·5
None (24 hr)	407 ± 35	4·2 ± 0·2
Thiosemicarbazone	117 ± 23	20·0 ± 4·2
Selenosemicarbazone	136 ± 28	21·6 ± 4·3
Guanylhydrazone	484 ± 39	3·5 ± 0·3
Semicarbazone	477 ± 39	3·4 ± 0·2

* Mice bearing 5-day implants of Sarcoma 180 ascites cells received a single i.p. dose of 20 mg/kg of the indicated drugs. Twenty-four hr later, cells were removed quantitatively from both untreated and drug-treated animals and counted with a Coulter model A particle counter. The volume occupied by a known number of cells was measured in hematocrit tubes. Each value represents the results obtained using 12 mice.

of the biochemical measurements were ascertained. The results are shown in Table 2. Administration of a single dose of 20 mg/kg of either 5-HP or its selenosemicarbazone on the fifth day after tumor implantation resulted in a pronounced decrease in tumor growth as expressed by the number of ascites cells present in the peritoneal cavity. The number of cells present in the abdominal cavities of mice treated with these two agents was significantly less than the quantity of ascites cells initially present, indicating that these agents were carcinolytic. Toxic reaction to these two compounds was also expressed as a pronounced increase in the average volume of treated cells. The guanylhydrazone and semicarbazone analogs showed no toxicity to the cells under these conditions.

TABLE 3. EFFECT OF 5-HYDROXY-2-FORMYLPYRIDINE THIOSEMICARBAZONE (5-HP) AND ITS ANALOGS ON THE INCORPORATION OF ^{3}H-THYMIDINE, 5-^{3}H-CYTIDINE AND 8-^{14}C-ADENINE INTO NUCLEIC ACIDS OF SARCOMA 180 ASCITES CELLS *in vitro**

Derivative of 5-hydroxy-2-formylpyridine	Concn (M)	^{3}H-thymidine DNA	5-^{3}H-cytidine RNA	5-^{3}H-cytidine DNA	8-^{14}C-adenine RNA	8-^{14}C-adenine DNA
DMSO (0·5%) control		− 2·7	− 3·4	−10·4	+ 6·9	− 1·8
Thiosemicarbazone	5×10^{-5}	−86·5	+ 2·6	−54·3	+13·7	−39·5
Selenosemicarbazone	1×10^{-5}	−25·2	− 7·1	−38·1	+10·1	−14·1
	5×10^{-5}	−80·2	− 2·2	−58·4	+ 3·7	−49·1
Guanylhydrazone	1×10^{-3}	−10·6	+11·2	−21·1	+26·7	− 9·1
Semicarbazone	1×10^{-3}	−14·9	+ 6·3	−33·0	+19·0	−16·4

* All data are average values of duplicate flasks from each of two experiments. The results are expressed as the per cent change in radioactivity present in the nucleic acids from non-DMSO-treated controls. Control specific activities are: thymidine into DNA = 4·4 cpm/nmole; cytidine into RNA = 51·0 cpm/nmole; cytidine into DNA = 5·0 cpm/nmole; adenine into RNA = 18·6 cpm/nmole; and adenine into DNA = 2·2 cpm/nmole.

The primary biochemical lesion caused by 5-HP *in vivo* has been shown to be blockade of the synthesis of DNA.[11] Inhibition of this cellular process is also demonstrable in intact cells *in vitro, as* shown in Table 3. Under these conditions 5-HP, at a concentration of 5×10^{-5} M, caused 86·5 per cent inhibition of incorporation of ^{3}H-thymidine into DNA of Sarcoma 180 ascites cells. The selenosemicarbazone analog at an equimolar concentration was almost equally effective, causing 80·2 per cent inhibition. The other two analogs, the guanylhydrazone and semicarbazone, were found to have no significant effect even at concentrations as great as 10^{-3} M.

The effects of various inhibitors on the incorporation of radioactivity from 5-^{3}H-cytidine and 8-^{14}C-adenine into RNA and DNA are also shown in Table 3. Both 5-HP and its selenosemicarbazone caused a significant decrease in the rate of incorporation of these radioactive tracers into the DNA of Sarcoma 180 ascites cells, while the utilization of these precursors for the formation of RNA was not depressed. The degree of inhibition of cytidine and adenine incorporation into DNA produced by these agents was considerably less than that measured by thymidine utilization for these macromolecules. The guanylhydrazone and semicarbazone analogs were considerably less potent as inhibitors of the synthesis of DNA, and required concentrations as great as 10^{-3} M to produce inhibition.

These findings indicated the requirement for either sulfur or selenium in the side chain of the molecule for effective inhibition of DNA synthesis and tumor growth.

TABLE 4. CONCENTRATION OF INHIBITORS REQUIRED TO CAUSE 50 PER CENT INHIBITION OF RIBONUCLEOSIDE DIPHOSPHATE REDUCTASE FROM NOVIKOFF RAT TUMOR

Derivative of 5-hydroxy-2-formylpyridine	ID_{50}* (M)
Thiosemicarbazone	$3{\cdot}5 \times 10^{-6}$
Selenosemicarbazone	$6{\cdot}8 \times 10^{-6}$
Guanylhydrazone	5×10^{-3}
Semicarbazone	5×10^{-3}

* The ID_{50} is the concentration of drug required to reduce by 50 per cent the observed activity of partially purified Novikoff rat tumor ribonucleoside diphosphate reductase under conditions described in Materials and Methods. The 50 per cent inhibitory concentrations were determined graphically from the results of at least two experiments with at least four doses of each compound.

The results correlated directly with concentrations of these derivatives required to produce 50 per cent inhibition of the activity of ribonucleoside diphosphate reductase from the Novikoff rat tumor (Table 4). The Novikoff hepatoma was employed as the enzyme source because it is more purified than the analogous preparation from Sarcoma 180; however, the degree of inhibition by 5-HP of the reduction of CDP to dCDP in cell-free preparations of Sarcoma 180 has been shown to be of the same magnitude as that of partially purified ribonucleoside diphosphate reductase prepared from the Novikoff rat tumor.[12] The thiosemicarbazone was the most active of the agents, requiring $3{\cdot}5 \times 10^{-6}$ M drug to cause 50 per cent inhibition of enzyme activity. The selenosemicarbazone required approximately twice this concentration to produce the same degree of inhibition, and the guanylhydrazone and semicarbazone were about 700 times less effective.

Indirect measurement of the activity of ribonucleoside diphosphate reductase *in situ*, by monitoring the passage of radioactivity from pyrimidine ribonucleotides to deoxyribonucleotides in whole cells of Sarcoma 180 incubated *in vitro* with 5-^{3}H-cytidine (Table 5), confirmed the findings obtained with the isolated enzyme. Both the thiosemicarbazone and selenosemicarbazone analogs were essentially equal in

TABLE 5. EFFECT OF 5-HYDROXY-2-FORMYLPYRIDINE THIOSEMICARBAZONE (5-HP) AND ITS ANALOGS ON THE INCORPORATION OF 5-^{3}H-CYTIDINE INTO ACID-SOLUBLE PYRIMIDINE DEOXYRIBONUCLEOTIDES*

Derivative of 5-hydroxy-2-formylpyridine	Concn (M)	% Inhibition
Thiosemicarbazone	5×10^{-5}	45·2
Selenosemicarbazone	5×10^{-5}	43·6
Guanylhydrazone	1×10^{-3}	18·4
Semicarbazone	1×10^{-3}	10·7

* All data are the average values of duplicate flasks from two experiments. The specific activity of control acid-soluble deoxyribonucleotides was 4030 cpm/g of cells.

activity in inhibiting the incorporation of 5-^{3}H-cytidine into acid-soluble deoxyribonucleotides, whereas the guanylhydrazone and semicarbazone were relatively ineffective. None of these agents had any effect on the incorporation of 5-^{3}H-cytidine into acid-soluble ribonucleotides.

Incorporation of D,L-leucine-l-^{14}C into total protein of Sarcoma 180 ascites cells treated with various derivatives *in vitro* was not decreased by concentrations of 5-HP or its selenosemicarbazone as great as 5×10^{-5} M, nor as great as 10^{-3} M guanylhydrazone or semicarbazone.

The ligand/metal binding ratios obtained with this series of chelating agents and the association constants for cobalt and copper are shown in Table 6. The cobalt to ligand binding ratio for 5-HP and its selenosemicarbazone analog was 1:2, supporting tridentate chelate formation. The guanylhydrazone and semicarbazone derivatives showed a 1:3 ratio with cobalt, implying that these analogs were chelating as bidentate ligands. Copper, which had a much lower affinity for the ligands than did cobalt, formed a 1:1 complex with 5-HP and the selenosemicarbazone, and a 1:2 complex with the guanylhydrazone and semicarbazone. The weakness of guanylhydrazone and semicarbazone interaction with both metals rendered Job's analysis[17] difficult to interpret and association constants could not be calculated with accuracy. 5-HP was the most effective ligand in each case, followed by the selenosemicarbazone; this chelating potential correlated with the biological and biochemical activities of the various agents.

TABLE 6. FORMATION OF METAL COMPLEXES BY 5-HYDROXY-2-FORMYLPYRIDINE THIOSEMICARBAZONE (5-HP) AND ITS ANALOGS WITH COBALT AND COPPER

Derivative of 5-hydroxy-2-formylpyridine	Cobalt		Copper	
	Metal/ligand	$K_{assoc.} \times 10^{-9}$	Metal/ligand	$K_{assoc.} \times 10^{-3}$
Thiosemicarbazone	1:2	56	1:1	19
Selenosemicarbazone	1:2	7·6	1:1	1
Guanylhydrazone	1:3		1:2	
Semicarbazone	1:3		1:2	

These direct relationships between tumor inhibition, interference with DNA biosynthesis and chelation potential may be in part explained by comparing the covalent radii and electronegativity of the various isosteres (Table 7). The covalent radius of sulfur, which was found to be the optimum effective substituent, is 1·04 Å. Selenium is about one-tenth larger (covalent radius, 1·17 Å); this change in size is accompanied not only by reduced chelating potency, but perhaps also by a distortion of the fit of the molecule on the enzyme binding site. Both N and O are much smaller atoms (covalent radii, 0·74 each), which presumably considerably limits effective binding. Electronegativity would be expected to be related in a reverse order because of the conjugated resonance of the side chain. Thus, the more electronegative atoms would be expected to produce lesser resonant forms for metal binding at the enzyme site.

In summary, it appears that 5-HP is the optimum member of this series with respect to anticancer activity, and that tumor-inhibitory potency is directly correlated

TABLE 7. SUMMARY OF THE PHYSICAL PROPERTIES OF THE SUBSTITUENT ELEMENTS OF THE VARIOUS ISOSTERES OF 5-HYDROXY-2-FORMYLPYRIDINE THIOSEMICARBAZONE (5-HP) AND ITS ANALOGS

Substituent element	Covalent radius (Å)	Electronegativity
S	1·04	2·5
Se	1·17	2·4
N	0·74	3·0
O	0·74	3·5

with chelating potential and inhibition of the synthesis of DNA. The site of action of these agents on the DNA biosynthetic pathways is at ribonucleoside diphosphate reductase, an iron-requiring biocatalyst.

Acknowledgements—We wish to thank Cheryl Vanderford, Florence Dunmore and Lynn Bon Tempo for their excellent assistance.

REFERENCES

1. E. J. BLANZ, JR. and F. A. FRENCH, *Cancer Res.* **28,** 2419 (1968).
2. K. C. AGRAWAL and A. C. SARTORELLI, *J. pharm. Sci.* **57,** 1948 (1968).
3. E. J. BLANZ, Jr., F. A. FRENCH, J. R. DOAMARAL and D. A. FRENCH, *J. med. Chem.* **13,** 1124 (1970).
4. K. C. AGRAWAL, B. A. BOOTH, R. L. MICHAUD, A. C. SARTORELLI and E. C. MOORE, *Proc. Am. Ass. Cancer Res.* **11,** 3 (1970).
5. W. A. CREASEY, K. C. AGRAWAL, R. L. CAPIZZI, K. K. STINSON and A. C. SARTORELLI, *Cancer Res.* **32,** 565 (1972).
6. J. J. COFFEY, S. A. KELLEY, P. E. PALM, J. A. R. MEAD and C. J. KENSLER, *Proc. Am. Ass. Cancer Res.* **13,** 44 (1972).
7. E. ETCUBANAS, C. TAN, N. WOLLNER, V. BETHUNE, I. KRAKOFF and J. BURCHENAL, *Proc. Am. Ass. Cancer Res.* **12,** 38 (1971).
8. R. C. DECONTI, B. R. TOFTNESS, K. C. AGRAWAL, R. TOMCHICK, J. A. R. MEAD, J. R. BERTINO, A. C. SARTORELLI and W. A. CREASEY, *Cancer Res.* **32,** 1455 (1972).
9. F. A. FRENCH and E. J. BLANZ, JR., *J. med. Chem.* **9,** 585 (1966).
10. R. W. BROCKMAN, R. W. SIDWELL, G. ARNETT and S. SHADDIX, *Proc. Soc. exp. Biol. Med.* **133,** 609 (1970).
11. B. A. BOOTH, E. C. MOORE and A. C. SARTORELLI, *Cancer Res.* **31,** 228 (1971).
12. E. C. MOORE, B. A. BOOTH and A. C. SARTORELLI, *Cancer Res.* **31,** 235 (1971).
13. A. C. SARTORELLI, K. C. AGRAWAL and E. C. MOORE, *Biochem. Pharmac.* **20,** 3119 (1971).
14. H. G. MAUTNER and W. D. KUMLER, *J. Am. chem. Soc.* **78,** 97 (1956).
15. R. HULS and M. RENSON, *Bull. Soc. chim. Belg.* **65,** 511 (1956).
16. R. L. MICHAUD and A. C. SARTORELLI, N 54, 155*th A.C.S. National Meeting*, San Francisco. Calif., March 31–April 5, 1968.
17. A. E. MARTELL and M. CALVIN, *Chemistry of the Metal Chelate Compounds*, pp. 29–32, Prentice-Hall, Englewood Cliffs, N. J. (1962).
18. K. C. AGRAWAL, B. A. BOOTH and A. C. SARTORELLI, *J. med. Chem.* **11,** 700 (1968).
19. E. C. MOORE, *Meth. Enzym.* **12,** 155 (1967).
20. J. R. ROBINSON, *Biochem. J.* **45,** 68 (1949).
21. B. A. BOOTH, T. E. DONNELLY, JR., A. ZETTNER and A. C. SARTORELLI, *Biochem. Pharmac.* **20,** 3109 (1971).