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Synthesis of Site-Directed Chelating Agents I: Pteridine Carboxaldehyde Thiosemicarbazones

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Abstract □ Several 6-formylpteridine thiosemicarbazones and semicarbazones were synthesized as potential inhibitors of the enzyme dihydrofolate reductase. The most active derivative in this series was 2,4-diaminopteridine-6-carboxaldehyde semicarbazone, which caused 50% inhibition of dihydrofolate reductase of human liver at a concentration of 1.4×10^{-7} *M*.

Keyphrases □ Pteridine carboxaldehyde thiosemicarbazones—synthesized and tested as site-directed chelating agents of dihydrofolate reductase □ 6-Formylpteridine thiosemicarbazones and semicarbazones—synthesized and tested as site-directed chelating agents of dihydrofolate reductase □ Dihydrofolate reductase—synthesis and testing of pteridine carboxaldehyde thiosemicarbazones as potential inhibitors

Thiosemicarbazones of heterocyclic carboxaldehydes with the formyl group alpha to a heteroaromatic ring nitrogen have been shown to possess both antineoplastic and antiviral activities (1–5); several different heteroaromatic ring systems are active carcinostatic agents with such potency correlating with the inhibition of the synthesis of DNA (6–8). The site of the metabolic lesion on the DNA biosynthetic pathways, as elucidated with 1-formylisoquinoline thiosemicarbazone and 2-formylpyridine thiosemicarbazone, two members of the most potent heterocyclic ring systems, is at the level of the conversion of ribonucleotides to deoxyribonucleotides (9–11). A study of the mechanism of action of 1-formylisoquinoline thiosemicarbazone and 2-formylpyridine thiosemicarbazone indicated that they inhibit the enzyme ribonucleoside diphosphate reductase either by binding to the target enzyme through chelation of iron in a metal-containing form of the enzyme or by initially forming an iron chelate of the inhibitors which interacts with the enzyme (9, 11).

In an effort to orient the chelating potential of the formyl thiosemicarbazone side chain positioned alpha to a heteroaromatic ring nitrogen atom to a vulnerable enzymatic site of neoplastic cells other than ribonucleoside diphosphate reductase, several pteridine thiosemicarbazones were synthesized. The pteridine nucleus was selected to direct the formyl thiosemicarbazone portion of the molecule to the enzyme dihydrofolate reductase. Although dihydrofolate reductase has not been shown to be a metal-containing enzyme, Hakala and Suolinna (12) showed that the enzyme from neoplastic cells is inactivated by the metal-chelating agents *o*-phenanthroline and ethylenediaminetetraacetate.

EXPERIMENTAL

Enzymatic Studies—Dihydrofolate reductase was prepared to homogeneity from human liver[1]. Enzymatic activity was assayed spectrophotometrically by measuring the decrease in absorbance at 340 nm. which results from the conversion of NADPH and dihydrofolate to $NADP^+$ and tetrahydrofolate (13).

2,4-Diamino-6-formylpteridine Semicarbazone—2,4-Diaminopteridine-6-carboxaldehyde (0.19 g.) was dissolved in 50 ml. of water containing 1 ml. of concentrated hydrochloric acid. Semicarbazide (0.1 g.) was dissolved separately in 10 ml. of water, and the two solutions were mixed and warmed for 15 min. Sodium acetate (1 g.) was added and the mixture was stirred. The resulting compound was filtered, washed with hot water and ethanol, and dried. The compound did not melt but slowly decomposed above 300°.

For analytical purposes, the compound was isolated as its hydrochloride salt.

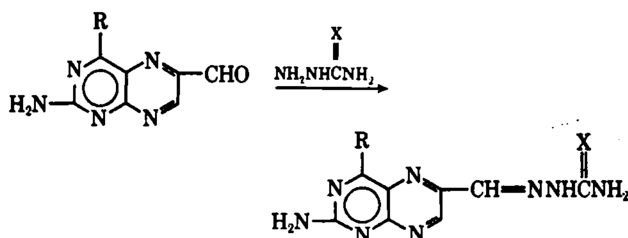
Anal.—Calc. for $C_8H_9N_9O \cdot HCl$: C, 33.86; H, 3.17; N, 44.44. Found: C, 33.36; H, 3.12; N, 43.95.

Thiosemicarbazone—The thiosemicarbazone derivative of 2,4-diaminopteridine-6-carboxaldehyde was synthesized as described above except that thiosemicarbazide replaced semicarbazide and was analyzed as its hydrochloride. The compound did not melt but slowly decomposed above 300°.

Anal.—Calc. for $C_8H_9N_9S \cdot HCl$: C, 32.05; H, 3.34; N, 42.07; S, 10.68. Found: C, 31.65; H, 3.30; N, 41.35; S, 10.49.

2-Amino-4-hydroxy-6-formylpteridine Semicarbazone—2-Amino-4-hydroxypteridine-6-carboxaldehyde (0.19 g.) was dissolved in 5 ml. of water by the addition of the minimum amount of 1 *N* sodium

[1] Performed by Dr. David R. Makulu and Dr. Joseph R. Bertino, who donated the enzyme for these studies.



I: $R = NH_2$, $X = O$
II: $R = NH_2$, $X = S$
III: $R = OH$, $X = O$
IV: $R = OH$, $X = S$

Scheme I

hydroxide. This solution was added dropwise to a hot solution of semicarbazide (0.1 g.) in 10 ml. of water containing a few drops of acetic acid, and the mixture was heated for a few minutes. The resulting compound was filtered, washed with hot water and ethanol, and then dried. It was purified by dissolving the compound in 1 *N* sodium hydroxide and reprecipitating with dilute acetic acid. The compound did not melt but slowly decomposed above 300°.

Anal.—Calc. for $C_8H_8N_8O_2 \cdot H_2O$: C, 36.09; H, 3.76; N, 42.10. Found: C, 35.71; H, 3.87; N, 42.17.

Thiosemicarbazone—The thiosemicarbazone derivative of 2-amino-4-hydroxypteridine-6-carboxaldehyde was synthesized as described above except that thiosemicarbazide replaced semicarbazide. The compound did not melt but slowly decomposed above 300°.

Anal.—Calc. for $C_8H_8N_8OS$: C, 36.36; H, 3.03; N, 42.42; S, 12.12. Found: C, 36.12; H, 3.21; N, 41.92; S, 11.90.

This paper reports the synthesis of the semicarbazones and thiosemicarbazones of 2,4-diaminopteridine-6-carboxaldehyde and 2-amino-4-hydroxypteridine-6-carboxaldehyde and the measurement of the potency of these compounds as inhibitors of dihydrofolate reductase from human liver. The pteridine aldehydes were synthesized by oxidative cleavage of folic acid (14) and aminopterin[2]; the resultant products were then reacted with semicarbazide or thiosemicarbazide (Scheme I).

RESULTS AND DISCUSSION

A comparison of the relative effectiveness of the pteridine carboxaldehyde thiosemicarbazones as inhibitors of the enzyme dihydrofolate reductase is shown in Table I. The results indicate that the 2,4-diaminopteridine derivatives are more potent than the corresponding 2-amino-4-hydroxypteridine compounds; these findings are in agreement with previous data indicating the requirement for 2,4-diamino groups for optimal inhibition of dihydrofolate reductase by pteridines. The best inhibitor of dihydrofolate reductase in this series was 2,4-diaminopteridine-6-carboxaldehyde semicarbazone (I), which caused 50% inhibition at a concentration of 1.4×10^{-7} *M*. Previous results from this laboratory (8, 15) indicated that the chelating potential of α-*N*-heterocyclic carboxaldehyde thiosemicarbazones is considerably greater than that of the corresponding semicarbazones; therefore, the findings indicate that chelating potential does not significantly enhance the activity of inhibitory pteridines.

[2] S. Kushner, Lederle Laboratories, Pearl River, N. Y., personal communication.

Table I—Effect of Pteridine Carboxaldehyde Thiosemicarbazones on Dihydrofolate Reductase Activity of Human Liver[a]

Compound	ID_{50}, *M*
2,4-Diaminopteridine-6-carboxaldehyde semicarbazone (I)	1.4×10^{-7}
2,4-Diaminopteridine-6-carboxaldehyde thiosemicarbazone (II)	7.5×10^{-7}
2-Amino-4-hydroxypteridine-6-carboxaldehyde semicarbazone (III)	1×10^{-6}
2-Amino-4-hydroxypteridine-6-carboxaldehyde thiosemicarbazone (IV)	1×10^{-6}

[a] Inhibitors were added to the enzymic reaction mixture in 10% dimethyl sulfoxide. Enzyme activity of controls as measured by the decrease in absorbance at 340 nm. was 0.187 absorbance unit/5 min. A concentration of 1.8×10^{-9} *M* methotrexate was required to inhibit enzyme activity by 50%.

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