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**Registry No.** 4, 87862-93-9; 5, 87862-94-0; 6, 87862-95-1; 7, 87862-96-2; 10, 34234-57-6; 11, 87862-97-3; 15, 87862-98-4; 18, 34017-27-1; 19, 76646-28-1; 20 [(*R*)-sulfoxide], 87862-99-5; 20 [(*S*)-sulfoxide], 87863-00-1; 21, 87863-02-3; 22, 87863-03-4; 23, 62305-89-9; 24, 20263-06-3; 1,1′-carbonyldiimidazole, 530-62-1; DBU, 6674-22-2; *S*-methylcysteine, 1187-84-4; benzyl chloroformate, 501-53-1; *O*-(mesitylenesulfonyl)hydroxylamine, 36016-40-7; dihydroorotase, 9024-93-5.

# Improved Synthesis and Antitumor Evaluation of 5,8-Dideazaisofolic Acid and Closely Related Analogues[1]

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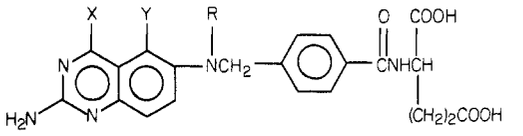
A new synthetic route to 5,8-dideazaisofolic acid (IAHQ) is described which precludes the possibility of contamination due to its 4-amino counterpart 5,8-dideazaisoaminopterin. Substitution of D-glutamic acid in this synthetic scheme gave D-IAHQ. The 9-formyl, 9-methyl, 5-methyl, and 5,9-dimethyl modifications of IAHQ were also prepared. These compounds, together with several structurally related or isomeric analogues, were studied for inhibitory effects upon the growth of four human gastrointestinal adenocarcinoma cell lines in vitro. In general, the compounds having a normal folate configuration at positions 9 and 10 are more active than their reversed bridge isomers. The lack of antitumor activity of D-IAHQ provides indirect evidence concerning the mechanism of action of IAHQ.

5,8-Dideazaisofolic acid (IAHQ, **1a**) was first described in 1975 as part of an ongoing synthetic program concerned with quinazoline analogues of folic acid.[2] Earlier studies had shown that its isomer 5,8-dideazafolic acid (AHQ), which has a normal folate configuration at positions 9 and 10, and the 10-$CH_3$ analogue (10-$CH_3$-AHQ) were effective inhibitors of thymidylate synthase from several different sources.[3–5] In vitro, 10-$CH_3$-AHQ inhibited the growth of mouse neuroblastoma cells, although not nearly as effectively as certain structurally related 2,4-diaminoquinazolines, which were shown to be potent inhibitors of dihydrofolate reductase (DHFR).[5]

Preliminary studies with IAHQ showed that at low doses using a single dose regimen on day 1 after tumor inoculation, the compound was ineffective against L1210 leukemia in mice.[2] IAHQ was a moderately effective inhibitor of rat liver DHFR, being some 10-fold less inhibitory than AHQ.[6] Subsequently, a large series of quinazoline analogues of folic acid was evaluated as inhibitors of thymidylate synthase from *Lactobacillus casei* and from L1210 leukemia cells.[7] IAHQ was found to be an effective inhibitor of the L1210 enzyme; however, AHQ and 10-$CH_3$-AHQ were significantly more inhibitory toward this enzyme. More recent studies revealed that IAHQ was an effective inhibitor of the growth of human colon adenocarcinoma cells (HCT-8) in vitro, thus generating interest in this compound for potential use in the treatment of methotrexate (MTX) unresponsive tumors.[8] Significant activity against colon tumor 38 in mice was also demonstrated, and when a regimen of 85 mg/kg on days 2 and 10 following tumor inoculation was used, there were 6 of 20 tumor-free animals after 90 days.[8] MTX was not effective in this model.[9] It was also found that IAHQ protected newborn hamsters from mortality due to transplantable human osteosarcoma cells, whereas MTX had no effect against this xenograph at the maximally tolerated dose.[10]

**Chemistry.** This paper describes a new unequivocal synthetic route to **1a**, which precludes the possibility of




**1a**, X = OH; Y = R = H
**b**, X = $NH_2$; Y = R = H
**c**, X = OH; Y = $CH_3$; R = H
**1d**, X = OH; Y = H; R = CHO
**e**, X = OH; Y = H; R = $CH_3$
**f**, X = OH; Y = $CH_3$; R = $CH_3$

trace contamination due to its 4-amino counterpart, 5,8-dideazaisoaminopterin (**1b**). The latter compound was shown to be a reasonably potent inhibitor of DHFR from rat liver.[6] Numerous earlier synthetic efforts employed diethyl esters of glutamate, which were removed in the final step under hydrolysis conditions using sodium hydroxide. During this study, the carboxyl groups of glutamic acid were protected by the use of *tert*-butyl esters, which are removed readily at ambient temperature under mildly acidic conditions. This modification yields final

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**Scheme I.** Improved Synthetic Route to IAHQ and Analogues

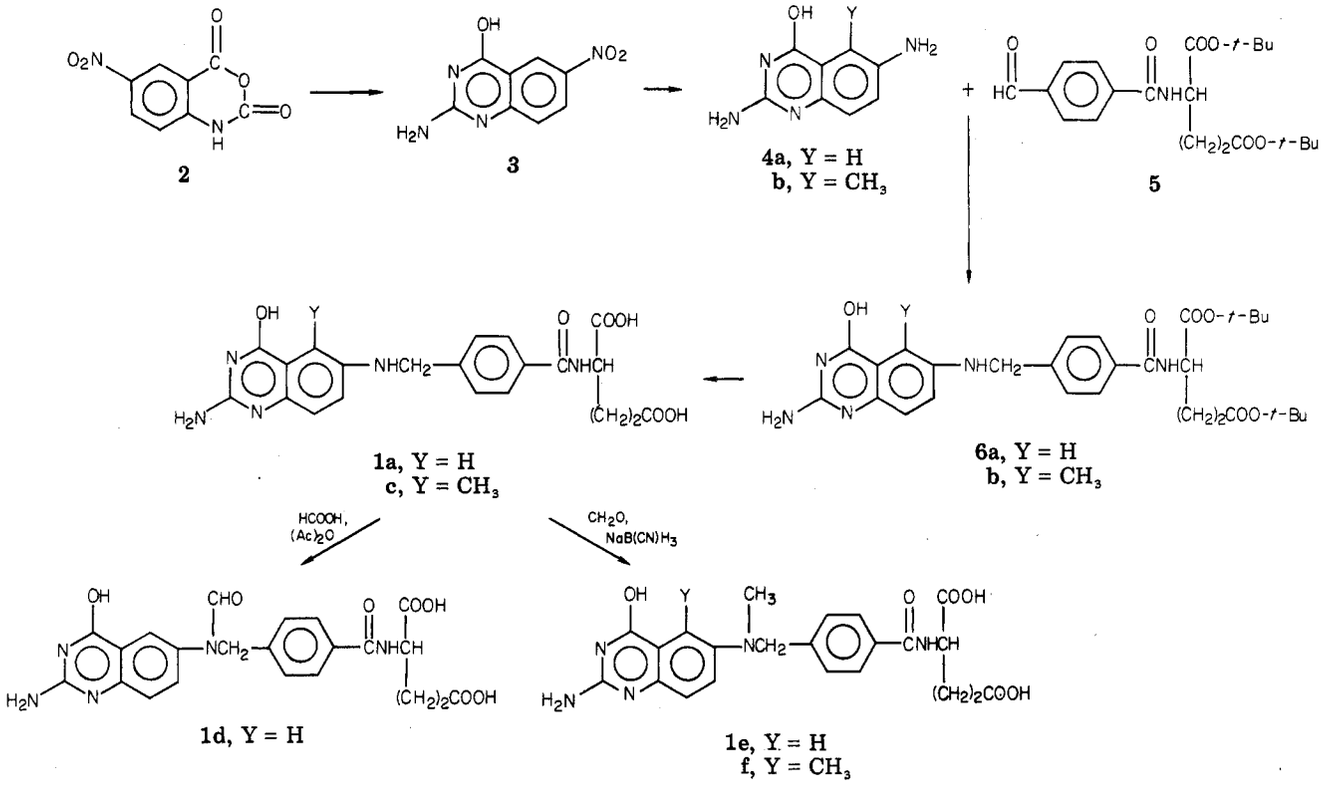


**Table I.** Inhibition of Growth of Human Gastrointestinal Adenocarcinoma Cells in Vitro by Selected Quinazoline Analogues of Folic Acid

| | growth inhibition: $IC_{50}$, μM | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| cell | IAHQ | D-IAHQ | AHQ | 9-CHO-IAHQ | 10-CHO-AHQ | 9-$CH_3$-IAHQ | 10-$CH_3$-AHQ | 5-$CH_3$-IAHQ | 5,9-$(CH_3)_2$-IAHQ | 1b | MTX |
| HuTu80 | 4.5 | >300 | 0.7 | 95 | 46 | 4.8 | 1.9 | 4.0 | 3.6 | 0.15 | 0.009 |
| HT29 | 1.7 | >300 | 0.6 | 100 | 38 | 3.2 | 1.2 | 1.1 | 2.7 | 0.20 | 0.018 |
| SW480 | 4.5 | >300 | 1.1 | 150 | 84 | 5.0 | 1.6 | 10 | 4.8 | 0.20 | 0.015 |
| WIDR | 4.2 | >300 | 0.6 | 195 | 41 | 6.3 | 1.3 | 4.0 | 4.8 | 0.20 | 0.025 |

products having a higher degree of purity and rules out the possibility of racemization occurring during the deprotection step. The steps employed are outlined in Scheme I. The key intermediate, 2,6-diamino-4-hydroxyquinazoline (**4**), was prepared in two steps from the commercially available 5-nitroisatoic anhydride [6-nitro-2*H*-3,1-benzoxazine-2,4(1*H*)-dione (**2**)]. Cyclization with guanidine carbonate, followed by reduction of the resulting nitroquinazoline using catalytic hydrogenation, yielded **4**. Compound **4** had formerly been derived by the acid hydrolysis of 2,4,6-triaminoquinazoline.[2] Di-*tert*-butyl L-glutamate was prepared by the acid-catalyzed reaction of L-glutamic acid with excess isobutylene under low pressure.[11] Subsequent coupling to 4-formylbenzoic acid using the mixed anhydride method gave di-*tert*-butyl *N*-(4-formylbenzoyl)-L-glutamate (**5**), which was obtained as a viscous oil. This was employed in succeeding transformations without further purification, since attempts to obtain **5** in solid form were unsuccessful. The reductive condensation of **4** with **5** in the presence of Raney nickel gave the di-*tert*-butyl ester of **1a**, **6**. After purification using silica gel chromatography, **6** was deprotected with anhydrous trifluoroacetic acid, affording **1a** in good yield. Substitution of D-glutamic for the natural isomer in the aforementioned sequence gave the D isomer, D-IAHQ. The reductive condensation of 2,6-diamino-4-hydroxy-5-methylquinazoline (**4b**) with **5** yielded the di-*tert*-butyl ester of the 5-methyl compound **6b**. Treatment with anhydrous trifluoroacetic acid then gave 5-$CH_3$-IAHQ (**1c**), which was found to have a greater degree of purity than that prepared earlier from diethyl L-glutamate, as determined by reverse-phase high-performance liquid chromatography.[2]

The reaction of **1a** with formic acid in the presence of acetic anhydride gave 9-CHO-IAHQ (**1d**) in respectable yield. The introduction of the 9-methyl group was accomplished according to the procedure employed for the methylation of folic acid, yielding 9-$CH_3$-IAHQ (**1e**).[12] Purification of this product required ion-exchange chromatography using DEAE-cellulose. The methylation of **1c** was effected in an analogous manner, giving 5,9-$(CH_3)_2$-IAHQ (**1f**). Isomeric compounds having a normal folate configuration at positions 9 and 10 were prepared as described elsewhere,[13] with the exception of 10-$CH_3$-AHQ. This compound was more readily prepared by direct

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methylation of AHQ rather than using the multistep sequence employed earlier.[14]

**Biological Evaluation.** Each of the target compounds, together with three isomeric analogues, having a normal folic acid configuration were evaluated for inhibitory activity upon the growth of four human gastrointestinal cell lines in vitro, and the results are presented in Table I. The 4-aminofolate analogues MTX and **1b** were included in order to compare relative antitumor potencies. It will be seen that each cell line was quite sensitive to MTX and that compound **1b** was approximately one order of magnitude less inhibitory. This correlates with the relative abilities of these two compounds to inhibit mammalian DHFR.[6] It is interesting that IAHQ, as well as its 5-$CH_3$, 9-$CH_3$, and 5,9-$(CH_3)_2$ derivatives, has approximately the same modest level of inhibitory activity toward these four cell lines. The 9-CHO derivative was considerably less active, while D-IAHQ was at least 100-fold less inhibitory than its L isomer. The di-*tert*-butyl ester **6a** was inactive within solubility limits. Both AHQ and its 10-$CH_3$ derivative were more cytotoxic than IAHQ. Since each of these was a better inhibitor of DHFR,[6] as well as of thymidylate synthase,[7] no conclusion can be drawn to explain this enhanced activity. As was the case for the formyl derivative of IAHQ, 10-CHO-AHQ was dramatically less inhibitory than AHQ. Since the latter compound was shown to be a good inhibitor of both DHFR[6] and thymidylate synthase,[7] it is suggested that the formyl modifications are not effectively transported into these cell lines.

## Discussion

Recent studies with IAHQ have indicated that its cytotoxicity may be related to intracellular conversion to poly($\gamma$-glutamyl) metabolites, which are significantly more inhibitory toward thymidylate synthase.[8] This inhibition was augmented by higher concentrations of 2′-deoxyuridine monophosphate, which accumulate as the result of inhibition of thymidylate synthase.[8] The formation of $\gamma$-L-glutamate metabolites would also be expected to result in enhanced retention of IAHQ within tumor cells, as has been observed for MTX in cultured human cells.[15] The inactivity of D-IAHQ may be attributable to the fact that it is not a substrate for folylpolyglutamate synthetase, as was the case for D-folic acid.[16] On the other hand, D-IAHQ may not be as effectively transported into these cells as IAHQ. Earlier studies with structurally related 2,4-diaminoquinazolines showed that a D-glutamic acid derivative had a much slower rate of influx into L1210 leukemia cells than its L isomer.[17] The wide divergence in activity between the two stereoisomers provides indirect evidence that racemization does not occur during the deblocking procedure. Additional studies using MTX-insensitive tumor cells both in vitro and in vivo will be required to determine whether IAHQ or one of its analogues will be of value in treating human neoplastic diseases.

## Experimental Section

Melting points were determined on a Mel-temp apparatus and are uncorrected. Elemental analyses were performed by Galbraith Laboratories, Knoxville, TN. All analytical samples were dried under vacuum at 100 °C unless stated otherwise and gave combustion values for C, H, and N within ±0.4% of the theoretical values. All intermediates were free of significant impurities on TLC using silica gel media (Gelman SAF or Baker 1B2-F). Target compounds (free glutamates) were analyzed by HPLC on a Micromeritics Model 7000B liquid chromatograph containing a Partisil PSX 10/25 ODS-2 column (4.6 × 25 cm) and a UV (254 nm) detector. The workup procedures described below were developed in such a manner that the final products were free of significant impurities using linear gradient elution (0–20% v/v) of MeCN in $H_2O$ at pH 4.0 and a flow rate of 2 mL/min. Samples for HPLC were dissolved in $Me_2SO$ just prior to injection. The UV spectra were determined with a Cary 219 spectrophotometer. The $^1H$ NMR spectra were determined with a Varian T-60 spectrometer operating at 60 MHz or a Varian EM 390 spectrometer operating at 90 MHz. Values for chemical shifts are presented in parts per million downfield from $Me_4Si$ as the internal standard. The relative peak areas are given to the nearest whole number, and chemical shifts in the case of multiplets are measured from the approximate center. Di-*tert*-butyl L-glutamate was prepared according to the literature method but was maintained in oil form rather than being converted into a salt derivative.[11] The free ester was found to be stable when stored at 4 °C: TLC ($CHCl_3$–MeOH–HOAc, 85:10:5). 5-Nitroisatoic anhydride was obtained from Sherwin Williams Chemicals and was recrystallized from EtOH–$H_2O$, 95:5, prior to use.

**2-Amino-4-hydroxy-6-nitroquinazoline (3).** A mixture of 16 g (0.077 mol) of **2** and 13.9 g (0.077 mol) of guanidine carbonate in 250 mL of DMF was heated at reflux with stirring for 48 h. The solution was added to 500 mL of $H_2O$ to give a solid, which was separated by filtration and washed with $(Me)_2CO$ and hexane to give **3**: yield 12.8 g (75%); TLC (DMF–EtOAc, 1:2). For analysis, a sample was recrystallized from $Me_2NAc$–$H_2O$ to yield a yellow powder: mp >350 °C dec; TLC (DMF–$H_2O$, 1:2); HPLC; NMR ($CF_3COOD$) $\delta$ 9.25 (d, 1, $H_5$, $J_{5,7}$ = 2 Hz), 8.33 (dd, 1, $H_7$, $J_{7,8}$ = 9 Hz, $J_{5,7}$ = 2 Hz), 8.03 (d, 1, $H_8$, $J_{7,8}$ = 9 Hz). Anal. ($C_8H_6N_4O_3 \cdot 0.1C_4H_9NO \cdot 0.8H_2O$) C, H; N: calcd, 25.05; found, 24.58. The presence of approximately 10% of $Me_2NAc$ was confirmed by NMR.

**2,6-Diamino-4-hydroxyquinazoline (4).** This compound was prepared by the literature method for the preparation of 2,4,6-triaminoquinazoline.[18] A 8.71 g (42.3 mmol) sample of crude **3** dissolved in 200 mL of DMF containing 2.6 mL (46.5 mmol) of glacial HOAc and 1 g of 10% Pd/C was hydrogenated at low pressure until $H_2$ uptake ceased. After filtration, the solution was poured into 1.4 L of EtOAc, and the resulting solid was collected by filtration and washed with $H_2O$ and $(Me)_2CO$. After drying in vacuo at 100 °C there was obtained 4.0 g (54%) of tan crystalline solid: mp 330–335 °C dec (lit.[19] mp >300 °C dec); TLC (DMF–$H_2O$, 1:2).

**Di-*tert*-butyl *N*-(4-Formylbenzoyl)-L-glutamate (5).** A solution of 20.3 g (0.135 mol) of 4-formylbenzoic acid and 13.6 g (0.135 mol) of 4-methylmorpholine in 200 mL of DMF was cooled to −15 °C, and the mixed anhydride was formed by the addition of 18.37 g (0.135 mol) of isobutyl chloroformate. After 2 min, a precooled (−15 °C) solution of 35.0 g (0.135 mol) of di-*tert*-butyl glutamate and 13.65 g (0.135 mol) of 4-methylmorpholine in 100 mL of DMF was added at one time. The mixture was stirred at −15 °C for 45 min and then allowed to warm to room temperature and stirred for 18 h. The solvent was removed at reduced pressure, and the residue was dissolved in 150 mL of EtOAc. This solution was washed with 3 × 50 mL of 5% $NaHCO_3$, 3 × 50 mL of 1 N $H_2SO_4$, and 3 × 50 mL of saturated NaCl. After the solution was dried over $Na_2SO_4$, the solvent was removed under vacuum, and the resulting oil was dried under vacuum until a constant weight of 45.7 g (81%) was obtained: TLC (EtOAc–MeOH, 98:2). Purification by silica gel column chromatography using $CHCl_3$ as the eluent yielded a pure fraction (TLC), which became contaminated, presumably due to oxidation during the solvent-removal step, and failed to crystallize: NMR ($CHCl_3$) $\delta$ 10.1 (s, 1, CHO), 7.92 (s, 4, aromatic), 4.67 (m, 1, NCH), 2.6–1.8 [m, 4, $(CH_2)_2$], 1.52 [s, 9, $OC(CH_3)_3$], 1.43 [s, 9, $OC(CH_3)_3$].

**Di-*tert*-butyl 5,8-Dideazaisofolate (6a).** A mixture of 8.0 g (19.0 mmol) of **5** and 3.75 g (19.1 mmol) of **4** in 160 mL of 70% HOAc was hydrogenated in the presence of Raney Ni (~500 mg)

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until $H_2$ uptake ceased. After the addition of charcoal, the catalyst was removed by filtration, and the filtrate was basified to pH 8 with $NH_4OH$. The precipitate was collected by filtration, washed with $H_2O$ and then hexane, and dried in vacuo over $P_2O_5$. The product was dissolved in 100 mL of $CHCl_3$ and washed with 2 × 100 mL of 10% $NaHSO_3$, 1 × 100 mL of $H_2O$, 2 × 100 mL of 5% citric acid, and 2 × 100 mL of $H_2O$ and then dried over $MgSO_4$. The solvent was removed under vacuum, and the product was washed with hexane and then dried in vacuo over $P_2O_5$. The product was purified by flash chromatography over Baker silica gel (40 μm), column 50 × 175 mm, using a stepwise gradient of $CHCl_3$ to $CHCl_3$/MeOH, 9:1: yield 5.5 g (52%); mp 145–147 °C; TLC ($CHCl_3$–MeOH, 85:15, Whatman $KC_{18}F$ RPTLC, MeOH/$H_2O$, 80:20). Anal. ($C_{29}H_{37}N_5O_6 \cdot 0.75H_2O$) C, H, N.

**5,8-Dideazaisofolic Acid (1a).** A 5.5 g (9.8 mmol) sample of **6a** was stirred in 80 mL of $CF_3COOH$ for 1 h at ambient temperature. The product was precipitated by the addition of excess $Et_2O$ and collected by centrifugation. The $Et_2O$–$CF_3COOH$ supernatant was decanted, and the product was washed 4 times with $Et_2O$ and then 2 times with $H_2O$. The product was suspended in $H_2O$, basified to pH 8.5 with $NH_4OH$, and then acidified to pH 4.0 with 0.5 N HCl to effect precipitation. The product was collected by filtration, washed with copious amounts of cold $H_2O$ and then $(Me)_2CO$, and finally dried in vacuo at 100 °C to yield 3.5 g (81.5%): mp >195 °C dec; TLC (0.1 M $NaHPO_4$, pH 7.5, cellulose); HPLC; UV $\lambda_{max}$ (0.1 M $NaHPO_4$, pH 7.0), 235 nm ($\epsilon$ 33.8 × $10^3$); NMR ($CF_3COOD$) δ 8.4–7.5 (m, 7, aromatic), 5.2–4.85 (m, 3, $NCH_2$ + NCH), 2.9–2.3 [m, 4, $(CH_2)_2$]. Anal. ($C_{21}H_{21}N_5O_6 \cdot 0.5H_2O$) C, H, N.

**D-5,8-Dideazaisofolic Acid (D-IAHQ).** Di-*tert*-butyl *N*-(4-formylbenzoyl)-D-glutamate was prepared in a manner identical with the preparation of its L isomer, **5**. The reductive condensation with **4**, followed by deblocking with $CF_3COOH$, then gave D-IAHQ. NMR, TLC, HPLC, and mp were identical with **1a**. Anal. ($C_{21}H_{21}N_5O_6 \cdot CF_3COOH \cdot 0.5H_2O$) C, H, N.

**Di-*tert*-butyl 5-Methyl-5,8-dideazaisofolate (6b).** A mixture of 9.57 g (24.5 mmol) of **5** and 4.65 g (24.5 mmol) of 2,6-diamino-4-hydroxy-5-methylquinazoline (**4b**)[2] in 200 mL of 70% HOAc was hydrogenated in the presence of Raney Ni (~500 mg) until $H_2$ uptake ceased. After addition of charcoal, the catalyst was removed by filtration, and the filtrate was basified to pH 8.5 with $NH_4OH$. The precipitate was collected by centrifugation, washed with 4 × 200 mL of $H_2O$, and then lyophilized. After drying over $P_2O_5$, there was obtained 7.74 g (54%): mp 139–141 °C; TLC (DMF–$H_2O$, 1:2, cellulose). Anal. ($C_{30}H_{39}N_5O_6 \cdot 2.25H_2O$) C, H, N.

**5-Methyl-5,8-dideazaisofolic Acid (1c).** A 5.6 g (9.2 mmol) sample of **6b** was treated with 80 mL of $CF_3COOH$ for 1 h, and the product was obtained as described for **1a**. There was obtained 2.45 g (56%): mp 253–254 °C (lit.[2] mp 220–225 °C); TLC (DMF–$H_2O$, 1:2, cellulose); HPLC; UV $\lambda_{max}$ (0.1 M $NaHPO_4$, pH 7.0) 240 nm ($\epsilon$ 40.0 × $10^3$); NMR ($CF_3COOD$) δ 8.08–7.42 (m, 6, aromatic), 5.17–4.80 (m, 3, $NCH_2$ + NCH), 2.98–2.30 [m, 7, $CCH_3$ + $(CH_2)_2$]. Anal. ($C_{22}H_{23}N_5O_6 \cdot 1.5H_2O$) C, H, N.

**9-Formyl-5,8-dideazaisofolic Acid (1d).** A 0.224 g (0.5 mmol) sample of **1a** was dissolved in a solution of 10 mL of HCOOH (97%), and 5 mL of $(Ac)_2O$ was added with stirring at room temperature. After 1 h, the product was precipitated by the addition of excess $Et_2O$, followed by chilling in an ice bath for 20 min. The precipitate was collected by filtration and washed with $H_2O$, then $(Me)_2CO$, and finally $Et_2O$. The product was dried at 100 °C in vacuo, yielding 0.182 g (68.5%): mp >210 °C dec; TLC (0.1 M $NaHPO_4$, pH 7.5, cellulose); HPLC; UV $\lambda_{max}$ (0.1 M $NaHPO_4$, pH 7.0) 230 nm ($\epsilon$ 44.5 × $10^3$); NMR ($CF_3COOD$) δ 8.3 (s, 1, CHO), 8.1–7.15 (m, 7, aromatic), 5.50–4.80 (m, 3, $NCH_2$ + NCH), 2.93–2.23 [m, 4, $(CH_2)_2$]. Anal. ($C_{22}H_{21}N_5O_7 \cdot 2H_2O$) C, H, N.

**9-Methyl-5,8-dideazaisofolic Acid (1e).** A 1.0 g (2.2 mmol) sample of **1a** was dissolved in 55 mL of 0.1 N NaOH and then neutralized to pH 6.4 with 1 N HCl. To this was added 1.0 g (12.7 mmol) of 38% $H_2CO$, followed by 0.20 g (3.18 mmol) of $NaB(CN)H_3$. The pH of the reaction mixture was maintained at 6.5 for 45 min by the occasional addition of 1 N HCl, and the reaction was continued for 20 h. The solution was then adjusted to pH 11 with 1 N NaOH, with stirring, and held at that point for approximately 10 min to allow HCN gas to escape. The solution was acidified to pH 4 with 1 N HCl to precipitate the product, which was collected by filtration, washed with $H_2O$ and then $Et_2O$, and dried at 100 °C in vacuo: yield 1.01 g (95%); mp >235 °C dec. Purification was effected by anion-exchange chromatography using a 2 × 20 cm column containing Cellex D (Bio-Rad). A linear gradient of 0.02–0.35 M $NH_4HCO_3$, pH 8.5, was used to elute the product. After lyophilization and neutralization to pH 4, the solid had mp >235 °C after vacuum drying at 100 °C: TLC (0.1 M $NaHPO_4$, pH 7.5, cellulose); HPLC; UV $\lambda_{max}$ (0.1 M $NaHPO_4$, pH 7.0) 235 nm ($\epsilon$ 38.6 × $10^3$); NMR ($CF_3COOD$) δ 8.54–7.48 (m, 7, aromatic), 5.13–4.83 (m, 3, $NCH_2$ + NCH), 3.6 (s, 3, $NCH_3$) 2.9–2.3 [m, 4, $(CH_2)_2$]. Anal. ($C_{22}H_{23}N_5O_6 \cdot 2.75H_2O$) C, H, N.

**5,9-Dimethyl-5,8-dideazaisofolic Acid (1f).** A 0.88 g (1.83 mmol) sample of **1c** was methylated and purified according to the procedures employed for **1e**. Following anion-exchange chromatography, a 69.5% yield was obtained: mp 223–225 °C; TLC (0.1 M $NaHPO_4$, pH 7.5, cellulose); HPLC; UV $\lambda_{max}$ (0.1 M $NaHPO_4$, pH 7.0) 235 nm ($\epsilon$ 39.2 × $10^3$); NMR ($CF_3COOD$) δ 8.32–7.30 (m, 6, aromatic), 5.25–4.80 (m, 3, $NCH_2$ + NCH), 3.65 (s, 3, $NCH_3$), 2.95–2.20 [m, 7, $CCH_3$ + $(CH_2)_2$]. Anal. ($C_{23}H_{25}N_5O_6 \cdot 1.5H_2O$) C, H, N.

**10-Methyl-5,8-dideazafolic Acid (10-$CH_3$-AHQ).** A 0.70 g (1.5 mmol) sample of 5,8-dideazafolic acid[14] was methylated by the same method described for **1e**: yield 0.59 g (84%); mp >220 °C dec (lit.[14] mp 220–225 °C dec); TLC (0.1 M $NaHPO_4$, pH 7.5, cellulose); HPLC; UV $\lambda_{max}$ (0.1 M $NaHPO_4$, pH 7.0) 230 nm ($\epsilon$ 44.5 × $10^3$); NMR ($CF_3COOD$) δ 8.35–7.45 (m, 7, aromatic), 5.22–4.90 (m, 3, $NCH_2$ + NCH), 3.62 (s, 3, $NCH_3$), 2.9–2.3 [m, 4, $(CH_2)_2$].

**Biological Evaluation.** Determinations of the growth of tumor cells in the presence of drugs were conducted in CoStar (Cambridge, MA) 24-well plates. Experiments were begun by the addition of 1 mL of media containing 0.7–1 × $10^5$ monodispersed growing cells to wells in which 0–50 μL of solution of the particular drug had been placed. Compounds were dissolved just prior to assay in 0.5 mL of 0.1 M NaOH and then diluted with 4.5 mL of phosphate buffer (pH 7.0). The cells were incubated at 37 °C in a humidified 8% $CO_2$ atmosphere for 72 to 96 h. The monolayers of cells were then removed by trypsinization and counted in suspension. Cell number was determined on a Coulter counter and maximally increased 10- to 15-fold over that inoculated.[20] $IC_{50}$ values refer to that concentration of inhibitor necessary to inhibit cell growth by 50% compared to control cells grown under identical conditions, except that the inhibitor was omitted. Standard deviations for all values presented in Table I, based upon four determinations, were less than 5%. The establishment of the cell lines employed in this study has been described previously: HuTu90,[21] HT29,[22] SW480,[23] and WIDR.[24]

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