# Synthesis of Quinazoline Analogs of Isofolic Acid[1]

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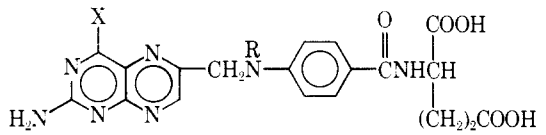
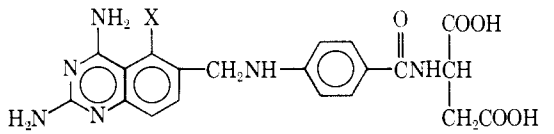
Four quinazoline analogs of isofolic acid were synthesized including 5-methyl-5,8-deazaisofolic acid (**8a**), 5,8-deazaisofolic acid (**8c**), as well as their 4-$NH_2$ counterparts **8b** and **8d**. None of these showed significant activity against L1210 leukemia in mice at dose levels where amethopterin provided significant prolongation in survival.

Numerous chemical modifications of folic acid (**1a**) have been synthesized and evaluated as potential antimetabolites. Active analogs which retain a terminal amino acid residue are referred to as classical folate antagonists and are usually targeted for cancer chemotherapy. The most significant achievement in this area involved the development and successful utilization of amethopterin (**1b**) in the treatment of choriocarcinoma, acute lymphoblastic leukemia, and Burkett's lymphoma.[2] A number of tumors, however, are naturally resistant to **1b**, while others become refractory despite initial sensitivity. Therefore, quinazoline analogs of **1a**, such as chlorasquin (**2a**) and methasquin (**2b**), have



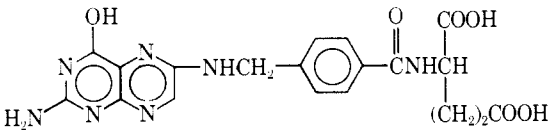
**1a**, X = OH; R = H
**b**, X = $NH_2$; R = $CH_3$

generated considerable interest.[3] This is due to the fact that in addition to displaying significant antitumor activity in vivo, **2b** showed only partial cross-resistance to bacterial



**2a**, X = Cl
**b**, X = $CH_3$

or Chinese hamster cell lines which were highly resistant to **1b**.[4-6] Recently, the synthesis and preliminary biologic evaluation of isofolic acid (**3**) were reported.[7] Against



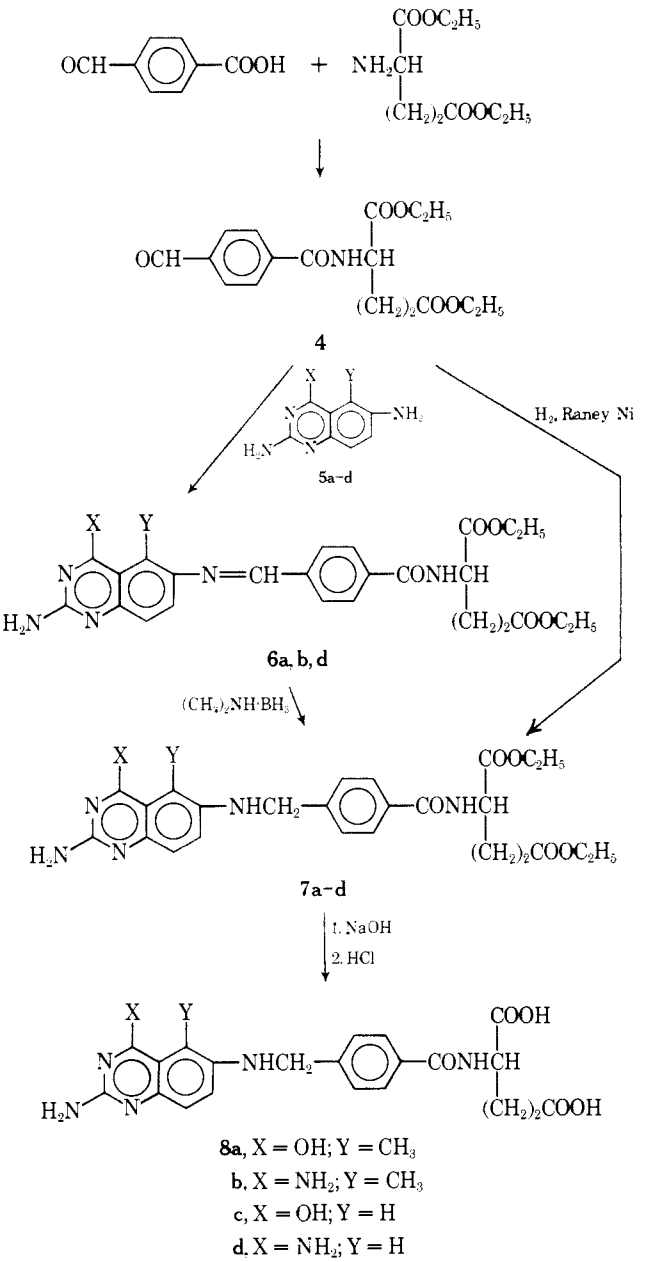
**3**

*Streptococcus faecium* in vitro, **3** was shown to display a similar level of growth inhibitory potency to that exhibited by **1b**. Furthermore, reversal studies implied that **3** was functioning primarily by inhibiting thymidylate synthetase.[7] Conversely, the primary site of action for **1b** as well as for **2a** and **2b** has been shown to be the enzyme dihydrofolate reductase. Therefore, we have synthesized a series of quinazoline analogs of **3** in the hope of obtaining compounds with selective inhibitory action upon thymidylate synthetase. It was anticipated that such compounds might prove to be effective against amethopterin-resistant tumors. In this study the amino acid residue was restricted to L-glutamyl since in earlier studies on quinazolines containing the normal folate bridge this moiety produced the most potent inhibitors of thymidylate synthetase from *Escherichia coli*.[8] It should be noted that isoquinespar, which differs from **8d** in that it contains an L-aspartyl moiety, was recently reported to be inactive against L1210 leukemia in mice.[9]

**Chemistry.** The target compounds for this study were 5-methyl-5,8-deazaisofolic acid (**8a**) and 5,8-deazaisofolic acid (**8c**). Their 4-$NH_2$ counterparts **8b** and **8d** were also synthesized in view of structural similarities to the known folate antagonists **1b**, as well as **2a** and **2b**. The physical properties of these compounds as well as their precursors are presented in Table I.

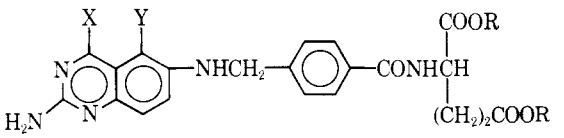
Synthetic routes employed in the preparation of **8a-d** are summarized in Scheme I. Condensation of diethyl L-

**Scheme I**



**8a**, X = OH; Y = $CH_3$
**b**, X = $NH_2$; Y = $CH_3$
**c**, X = OH; Y = H
**d**, X = $NH_2$; Y = H

**Table I.** Properties of Quinazoline Analogs of Isofolic Acid



| No. | X | Y | R | Mp, °C | Yield, % | Method[a] | Formula[b] | Recrystn medium | $I_{50}$, $\mu M$[c] |
|---|---|---|---|---|---|---|---|---|---|
| **6a**[d] | OH | $CH_3$ | $C_2H_5$ | 182–186 dec | 63 | A | $C_{26}H_{29}N_5O_6$ | | |
| **6b**[d] | $NH_2$ | $CH_3$ | $C_2H_5$ | 167–169 | 51 | B | $C_{26}H_{30}N_6O_5 \cdot 1.5H_2O$ | EtOH | |
| **6d**[d] | $NH_2$ | H | $C_2H_5$ | 150–155 | 77 | B | $C_{25}H_{28}N_6O_5 \cdot 1.5H_2O$ | | |
| **7a** | OH | $CH_3$ | $C_2H_5$ | 156–159 | 68 | C | $C_{26}H_{31}N_5O_6$ | MeOH | 2.7 |
| **7b** | $NH_2$ | $CH_3$ | $C_2H_5$ | 104–105 | 80 | C | $C_{26}H_{32}N_6O_5 \cdot H_2O$ | | 0.020 |
| **7c** | OH | H | $C_2H_5$ | 121–123 | 93 | D | $C_{25}H_{29}N_5O_6$ | | 0.57 |
| **7d** | $NH_2$ | H | $C_2H_5$ | 97–98 | 58 | D | $C_{25}H_{30}N_6O_5 \cdot 2H_2O$[e] | MeOEtOH–$Et_2O$ | 0.029 |
| **8a** | OH | $CH_3$ | H | 220–225 | 57 | E | $C_{22}H_{23}N_5O_6 \cdot 1.5H_2O$ | | 0.39 |
| **8b** | $NH_2$ | $CH_3$ | H | 235–240 dec | 71 | E | $C_{22}H_{24}N_6O_5$ | | 0.0056 |
| **8c** | OH | H | H | 179–181 dec | 72 | E | $C_{21}H_{21}N_5O_6 \cdot 0.5H_2O$ | | 0.21 |
| **8d** | $NH_2$ | H | H | 247–249 dec | 27 | E | $C_{21}H_{22}N_5O_5 \cdot 0.5H_2O$ | | 0.026 |

[a] See Experimental Section. [b] Anal. C, H, N. Several of these compounds analyzed correctly as hydrates or fractional hydrates even after drying in vacuo at 100°. Similar findings have been reported with related 2,4-diaminoquinazolines.[3] [c] Concentration required to produce 50% inhibition of rat liver dihydrofolate reductase. Assayed spectrophotometrically (340 mμ) with 9 $\mu M$ dihydrofolate, 30 $\mu M$ NADPH, and 0.15 $M$ KCl in 0.05 $M$ Tris buffer (pH 7.4). Under these conditions the value for pyrimethamine was 0.07 $\mu M$.[14] [d] Anil. [e] N: calcd, 15.84; found, 14.43. Compound is hygroscopic and difficult to dry due to low melting point.

glutamate with 4-formylbenzoic acid in the presence of dicyclohexylcarbodiimide proceeded slowly yielding the desired product, diethyl 4-formylbenzoyl-L-glutamate (**4**) together with an unidentified substance and dicyclohexylurea. Since this mixture could not be resolved by conventional recrystallization techniques, it was necessary to employ column chromatography on silica gel in order to obtain compound **4** in a highly purified state.

The requisite amines, 5-methyl-2,4,6-triaminoquinazoline (**5b**) and 2,4,6-triaminoquinazoline (**5d**), were prepared according to the methods of Davoll and Johnson.[3] Acid-catalyzed hydrolysis of these yielded respectively 2,6-diamino-4-hydroxy-5-methylquinazoline (**5a**) and the previously reported compound 2,6-diamino-4-hydroxyquinazoline (**5c**).[10] Two routes were employed in preparing the diethyl glutamate derivatives **7a–d.** The first involved the condensation of **4** with **5a**, **5b**, or **5d** to produce the corresponding anils **6a**, **6b**, and **6d**, the first two of which were subsequently reduced with dimethylamine borane[11] in acetic acid to **7a** and **7b.** Alternatively, the reductive condensation of **4** with **5c** and **5d** yielded **7c** and **7d** directly. Finally, saponification of the diethyl esters with dilute base afforded the free glutamic acid derivatives **8a–d.**

**Biological Results.** Each of the free glutamate derivatives **8a–d** was tested against L1210 leukemia in mice at 5 and 25 mg/kg.[12] Under these conditions **1b** produced increases in survival time of 33 and 68%, respectively. None of the newly synthesized compounds afforded a statistically significant increase (>25%) at either dose level.

Each of the target compounds **8a–d**, as well as the corresponding diethyl esters **7a–d**, was also evaluated as an inhibitor of rat liver dihydrofolate reductase in vitro. These results are included in Table I. It will be seen that the 4-OH derivatives are only moderately effective inhibitors with the free glutamates **8a** and **8c**, being somewhat more effective than the esters **7a** and **7c.** The presence of a 5-$CH_3$ group results in a modest decrease in activity for these compounds. The presence of a 4-$NH_4$ group causes an increase in inhibitory potency of from 10- to 100-fold. The most effective inhibitor, **8b**, is four times more effective than **8d** which is devoid of a methyl group at position 5. It is also more effective than its diethyl ester **7b.** On the other hand, hydrolysis of **7d** to yield **8d** does not significantly alter inhibitory activity. In view of the potent inhibition of dihydrofolate reductase in vitro caused by **8b** coupled with its inactivity against the experimental tumor, insufficient transport into leukemic cells is implied. Additional studies are now in progress which are designed to clarify this situation.

## Experimental Section

All analytical samples were dried under vacuum at 100° unless otherwise stated and gave combustion values for C, H, and N within ±0.4% of the theoretical values. Because of their low melting points **7b** and **7d** were dried over refluxing $CHCl_3$. Melting points were obtained with a Mel-Temp or a Fisher-Johns apparatus and are uncorrected. Thin-layer chromatographic analyses were performed on silica gel media (Gelman SAF). Dicyclohexylcarbodiimide (DCC) and 4-formylbenzoic acid were obtained from Aldrich Chemical Co., while diethyl L-glutamate hydrochloride was purchased from Sigma Chemical Co. Representative examples are presented for each of the synthetic methods designated in Table I.

**Diethyl 4-Formylbenzoyl-L-glutamate (4).** Equimolar amounts (0.045 mol) of 4-formylbenzoic acid (6.76 g), DCC (9.28 g), and diethyl L-glutamate (10.8 g) were dissolved in 300 ml of pyridine. The resulting mixture was stirred at ambient temperature in the absence of moisture for 7 days. The TLC (MeCN) showed the presence of a slower moving contaminant in addition to the product. The resulting dicyclohexylurea was separated by filtration and the pyridine was removed in vacuo. The yellow oil was dissolved in $CHCl_3$ (100 ml) and a small amount of insoluble material removed by filtration. Next, the solution was washed four times with 50-ml portions of 1 $N$ HCl, 5% $NaHCO_3$, and $H_2O$ and then dried over $MgSO_4$. The $CHCl_3$ was removed in vacuo and the residue dissolved in 50 ml of benzene. This solution was poured through a 2 × 50 cm column packed with 60–100 mesh silica gel. The product was eluted with benzene–MeOH (98:2) as monitored by TLC. The eluent was removed in vacuo and the product crystallized by trituration with isomeric hexanes. The off-white crystalline solid was separated by filtration, washed with hexanes, and dried in vacuo at 60° to yield 6.5 g (43%): mp 74–76° (TLC in MeCN). Anal. ($C_{17}H_{21}NO_6$) C, H, N.

**2,6-Diamino-4-hydroxy-5-methylquinazoline (5a).** A mixture of 5.68 g (0.03 mol) of 5-methyl-2,4,6-triaminoquinazoline (**5b**), prepared according to the method of Davoll and Johnson,[3] and 100 ml of 2 $N$ HCl was heated at reflux for 4.5 hr. The mixture was allowed to cool and then made basic by the addition of concentrated

$NH_4OH$. The resulting yellow precipitate was isolated by filtration and then recrystallized from DMF–$H_2O$ containing excess $NH_4OH$ (charcoal). The crystals were collected on a filter, washed with acetone, and dried yielding 2.5 g: mp 332–334°. The filtrate was concentrated to 33% of the initial volume in vacuo and then made basic with concentrated $NH_4OH$. The resulting precipitate was separated and then recrystallized as above yielding 1.3 g: mp 332–334°. These two crops were combined yielding a total of 3.8 g (58%) (TLC in DMF). Anal. ($C_9H_{10}N_4O \cdot 1.5H_2O$) C, H, N.

**Method A. Diethyl *N*-[α-(2-Amino-4-hydroxy-5-methylquinazol-6-ylimino)-*p*-toluyl]-L-glutamate (6a).** A 2.85-g (0.015 mol) sample of **5a** and 5.53 g (0.0165 mol) of **4** were heated at reflux in DMSO for 6 hr in the presence of 1 g of 4Å molecular sieves. After cooling and filtering, $H_2O$ was added to effect precipitation. The product was isolated by filtration, washed with $H_2O$, and then dried in vacuo, yielding 4.8 g (63%) (TLC in 1:4 DMF–EtOAc).

**Method B (6b and 6d).** Each of these was prepared in a similar manner except that in the case of **6b** only a 10% excess of aldehyde **4** was employed and a lower yield was obtained.

A mixture of 2.26 g (6.7 mmol) of **4**, 0.80 g (4.5 mmol) of 2,4,5-triaminoquinazoline[3] (**5d**), and 1 g of 4Å molecular sieves was boiled in 100 ml of EtOH for 18 hr. The TLC (DMF) showed that incomplete conversion to the anil had occurred. Therefore, 0.8 g (2.4 mmol) of additional **4** was added and the boiling continued for an additional 24 hr. After filtering the reaction mixture was refrigerated, causing the precipitation of a finely divided yellow solid. This was separated by filtration, washed with EtOH, and then dried in vacuo to yield 1.8 g (77%) of **6d** (TLC in DMF).

**Method C (7a and 7b).** These reductions were run in an identical manner except that heating was not required in the case of **7b**.

To a suspension of 2.54 g (5 mmol) of **6a** in 10 ml of glacial HOAc was added slowly with stirring 0.44 g (7.5 mmol) of dimethylamine borane in 10 ml of glacial HOAc. After stirring at ambient temperature for 2 days the reaction appeared to be incomplete by TLC so the mixture was heated to ca. 50° for 1 hr. Next, 10 ml of $H_2O$ was added and the solution was neutralized to pH 8 with concentrated $NH_4OH$. The resulting solid was separated by filtration, washed with $H_2O$, and then dried in vacuo. Recrystallization from MeOH yielded 1.7 g (68%) of yellow crystalline **7a** (TLC in DMF–EtOAc, 1:4).

**Method D (7c and 7d).** A mixture of 1.5 g (8.5 mmol) of 2,6-diamino-4-hydroxyquinazoline[10] (**5c**) and 3.2 g (9.5 mmol) of **4** in 100 ml of 70% aqueous HOAc was hydrogenated in the presence of Raney nickel until $H_2$ uptake had ceased. TLC at this point showed that all of the quinazoline had been consumed. The solution was treated with charcoal, filtered through Celite, and then basified with concentrated $NH_4OH$ to pH 8. The precipitate was separated by filtration, washed with $H_2O$, and then dried in vacuo yielding 3.9 g (93%) of **7c** as a yellow powder (TLC in DMF–MeCN, 1:3).

**Method E (8a–d).** Each of the hydrolysis reactions was conducted in a similar manner except that in the case of **7d**, EtOH (20%) was employed in order to improve solubility.

A suspension of 1.8 g (3.6 mmol) of **7c** in 144 ml of 0.1 *N* NaOH was stirred at ambient temperature for 3 days. Dissolution occurred gradually. The resulting solution was treated with charcoal, filtered through Celite, and then neutralized to pH 4.5 with 0.1 *N* HCl. The precipitate was isolated by filtration, washed with $H_2O$ and acetone, and then dried in vacuo. There was obtained 1.1 g (72%) of **8c** as a light tan solid (TLC in DMF).

## References and Notes


(1) This research was supported by NIH General Research Support R.R. 05420 to the Medical University of South Carolina and by a Summer Student Research Grant from the College of Graduate Studies, Medical University of South Carolina (C.M.G.).
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# Synthesis and Biologic Evaluation of Major Metabolites of *N*-(2-Chloroethyl)-*N'*-cyclohexyl-*N*-nitrosourea

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*N*-(2-Chloroethyl)-*N'*-(*cis*-4-hydroxycyclohexyl)-*N*-nitrosourea, a major metabolite of *N*-(2-chloroethyl)-*N'*-cyclohexyl-*N*-nitrosourea (CCNU), and its trans isomer were prepared from the corresponding 4-aminocyclohexanols. A convenient and stereospecific precursor was found in 2-oxa-3-azabicyclo[2.2.2]oct-5-ene hydrochloride, hydrogenation giving pure *cis*-4-aminocyclohexanol hydrochloride. The metabolites were, at nontoxic levels, at least as active as CCNU in tests against murine leukemia L1210 implanted both intraperitoneally and intracerebrally and, on a weight basis, were more active and more toxic. These observations and previously reported metabolic studies suggest that the anticancer activity of CCNU is due primarily to its metabolites.

*N*-(2-Chloroethyl)-*N'*-cyclohexyl-*N*-nitrosourea (CCNU) is highly active against leukemia L1210 in mice and has proven to be a useful drug in man.[1] It has recently been shown that CCNU is rapidly metabolized by liver microsomal material from mice without loss of the cytotoxic nitrosoureido function,[2] raising the possibility that the anticancer activity of this agent is due primarily to its metabolites rather than to the drug itself. The major metabolite in mice has been identified as *N*-(2-chloroethyl)-*N'*-(*cis*-4-hydroxycyclohexyl)-*N*-nitrosourea[2] (**7**), although at least three other ring-hydroxylated derivatives are also formed.[3] Only two metabolites, **7** and the *trans*-4-hydroxy derivative **2**, have been identified in the urine of human patients treated with CCNU.[4] These results made the synthesis,