A New Synthetic Route to Quinazoline Analogs of Folic Acid

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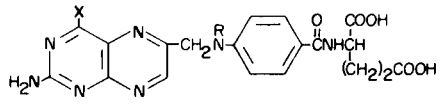
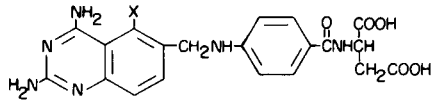
Close structural modifications of folic acid (**1a**) in which a quinazoline nucleus replaces the pteridine portion of the molecule have been studied extensively as potential chemotherapeutic agents. It was anticipated that compounds of this type might overcome certain of the therapeutic limitations of methotrexate (**1b**), which has been used with varying degrees of success in the treatment of choriocarbinoma, acute lymphoblastic leukemia, and Burkitts's lymphoma



1a, X = OH, R = H
b, X = NH_2, R = CH_3

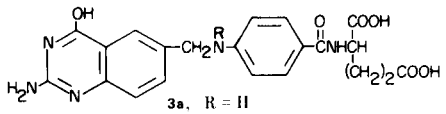
(1,2). Among the classical 2,4-diaminoquinazolines, methasquin (**2a**) and chlorasquin (**2b**) have demonstrated the greatest promise as antineoplastic agents (3,4,5).



2a, X = CH_3
b, X = Cl

However, limited clinical trials with **2a** showed no decided advantage over **1b** (6). Therefore, it would appear that these compounds will be relegated to the status of investitational tools.

The 2-amino-4-hydroxyquinazolines such as **3a** and **b**, which may be considered as 5,8-deazafolates, have received only limited study (3,7). This is due in part to their



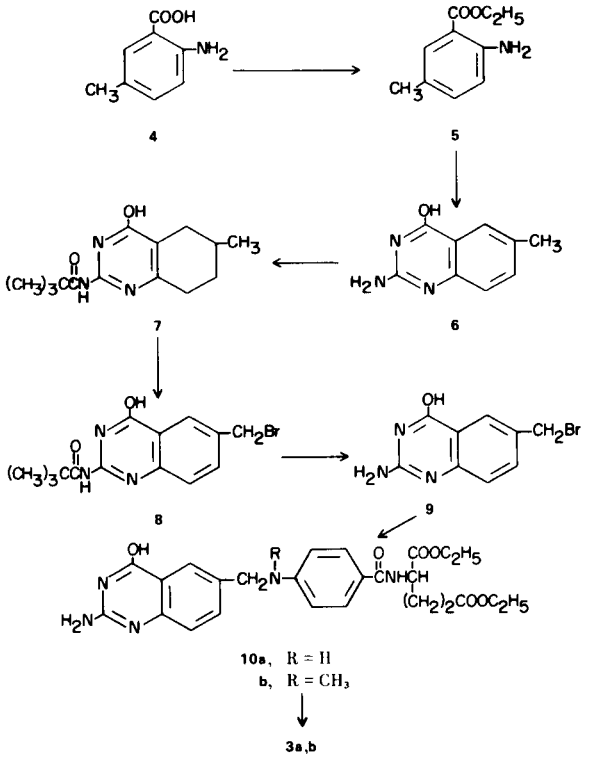
3a, R = H
b, R = CH_3

synthetic inaccessibility. In addition, *in vitro* studies have been hampered by the fact that in some instances samples of **3b** were contaminated with its 4-amino counterpart (8). Nevertheless, these compounds have been shown to be effective inhibitors of thymidylate synthetase from *Escherichia coli* (9), *Diplococcus pneumoniae* (10), and more importantly mouse neuroblastoma cells (8). It has been suggested, moreover, that a selective inhibitor of this enzyme could prove to be of value in producing "thymineless death" in cancer cell (11). This paper describes a new synthetic route which was designed to afford a variety of quinazoline analogs of folic acid free of contamination by closely related compounds. The approach employed is outlined in Scheme 1.

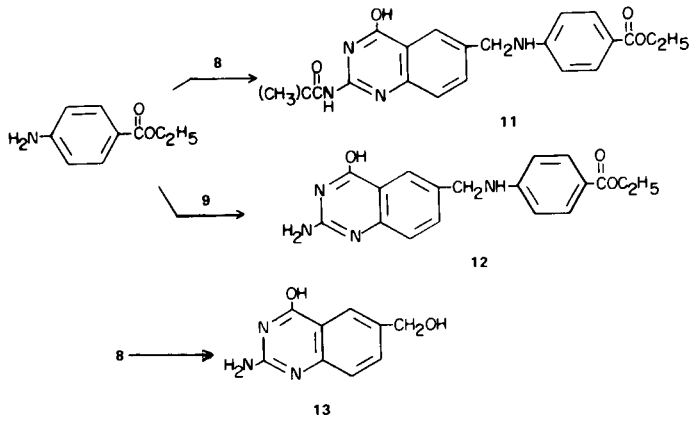
Although 2-amino-4-hydroxy-6-methylquinazoline, (**6**), has been reported previously (12,13), a more convenient and economical procedure was developed for this project. The acid catalyzed esterification of 2-amino-5-methylbenzoic acid (**4**) and subsequent cyclization of the resulting ester **5** with guanidine afforded **6** in 61% average overall yield. The direct bromination of **6** with either *N*-bromosuccinimide (NBS) or bromine proved unsuccessful. Therefore, protective acylation was conducted with trimethylacetyl chloride to yield **7**, which was found to have reasonable solubility in carbon tetrachloride. This derivative was readily brominated to yield the bromomethyl compound **8** using either NBS or 1,3-dibromo-5,5-dimethylhydantoin. Subsequent acid hydrolysis gave the key intermediate, 2-amino-6-bromomethyl-4-hydroxyquinazoline (**9**) in excellent yield. This compound in turn was alkylated with diethyl 4-aminobenzoyl-L-glutamate or diethyl *N*-methyl-4-aminobenzoyl-L-glutamate to yield **10a** or **10b**, respectively. However, it was necessary to employ column chromotography in order to obtain these compounds in analytical purity. Finally, saponification in dilute base afforded the desired 5,8-deazafolic acid analogs **3a** and **b**.

Some additional reactions of the bromomethyl compounds **8** and **9** are summarized in Scheme 2. Both reacted smoothly with ethyl 4-aminobenzoate to afford the pteroic acid analogs **11** and **12**. The latter compound was shown to be identical with a sample of this compound prepared by the reductive condensation of 2-amino-4-hydroxy-6-cyanoquinazoline with ethyl 4-aminobenzoate (14).

Scheme 1



Scheme 2



Finally, hydrolysis of **8** gave 2-amino-4-hydroxy-6-hydroxymethylquinazoline (**13**), thus providing a much simplier route to this compound than had formerly been described (15).

EXPERIMENTAL

Melting points were determined on a Fisher-Johns or a Mel-Temp apparatus and are uncorrected. Elemental analyses were performed by Galbraith Laboratories, Knoxville, Tennessee. Analytical samples were dried *in vacuo* at 100° and were free of significant impurities on tlc (Gelman SAF). All intermediates and target compounds had ir (Beckman IR-8) and nmr (Varian T-60) spectra in accord with their assigned structures. Values for chemical shifts deemed critical to structural assignments are presented in ppm (δ) downfield from TMS as an internal standard.

Ethyl 2-Amino-5-methylbenzoate (**5**).

Ethanol (800 ml.) was cooled in ice and saturated with hydrogen chloride gas. To it was added 160 g. (1.06 moles) of 2-amino-5-methylbenzoic acid and the mixture was heated at reflux for 18 hours. An almost clear solution resulted. Additional hydrogen chloride gas was added and the reflux was continued for 5 hours. The hot solution was poured into ice-water (1500 ml.), neutralized with sodium bicarbonate, and then extracted with ether. The ether extract was dried over magnesium sulfate and then evaporated *in vacuo*. The residue was distilled at 108-110°/1.5 mm. to yield 140-147 g. (74-77.5%) of **5**.

Anal. Calcd. for $C_{10}H_{13}NO_2$: C, 67.10; H, 7.31; N, 7.85. Found: C, 66.93; H, 7.36; N, 7.71.

2-Amino-4-hydroxy-6-methylquinazoline (**6**).

Sodium (16 g., 0.7 mole) was dissolved in 2-ethoxyethanol (200 ml.) with cooling. This solution was added with stirring to a solution of guanidine hydrochloride (70 g., 0.735 mole) in 2-ethoxyethanol (200 ml.) and stirred at room temperature for 10 minutes. The resulting solution was filtered and the solid was washed with 2-ethoxyethanol (2 x 50 ml.). These washings were added to the main filterate followed by 100 g. (0.56 mole) of compound **5**. The 2-ethoxyethanol was removed *in vacuo* and the residue was heated at 170-175° with stirring until solidification was complete and the heating was continued for 30 minutes more. The flask was cooled to 80° and ethanol was added with stirring. The product was isolated by filtration and washed with ethanol until the washings were colorless, yield, 78-82 g. (79-82%), m.p. 435-438° dec., with preliminary softening around 360° lit. (13) m.p. 439-440° dec.; nmr (deuteriotrifluoroacetic acid): δ 2.10 (s, 3, CH_3), 7.0 (d, 1, $J_{7,8}$ = 4 Hz, H-8), 7.45 (dd, 1, $J_{7,8}$ = 4 Hz, $J_{5,7}$ = 1 Hz, H-7), 7.75 (s broad, 1, H-5).

4-Hydroxy-6-methyl-2-trimethylacetamidoquinazoline (**7**).

A mixture of **6** (128 g., 0.73 mole), triethylamine (230 ml.), and *p*-dioxane (1200 ml.) was heated to reflux under stirring. To this mixture was added trimethylacetyl chloride (225 g., 1.85 moles) in *p*-dioxane (200 ml.). After addition, the heating was continued for an additional 0.5 hour. The hot reaction mixture was filtered and the solid was washed with hot *p*-dioxane. The filtrate combined with washings was cooled to room temperature and refiltered. The filtrate was concentrated *in vacuo* to a thick slurry and ethanol was added. The solid was isolated by filtration, washed with ethanol, ether, and then recrystallized from ethanol, yield, 140-144 g. (74-76%), m.p. 215-218°; nmr (deuteriotrifluoroacetic acid): δ 1.05 (s, 9, *t*-butyl), 2.15 (s, 3, CH_3), 7.15 (d, 1, $J_{7,8}$ = 4 Hz, H-8), 7.5 (dd, 1, $J_{7,8}$ = 4 Hz, $J_{5,7}$ = 1 Hz, H-7), 7.79 (s, broad, 1, H-5).

Anal. Calcd. for $C_{14}H_{17}N_3O_2$: C, 64.84; H, 6.69; N, 16.20. Found: C, 64.51; H, 6.80; N, 16.39.

6-Bromomethyl-4-hydroxy-2-trimethylacetamidoquinazoline (**8**).

A mixture of **7** (26 g., 0.1 mole) and NBS (18.5 g., 0.105 mole), which was purified by refluxing in carbon tetrachloride just prior to use, or 1,3-dibromo-5,5-dimethylhydantoin (15 g., 0.0525 mole) and carbon tetrachloride (800 ml.) was heated to a vigorous reflux. Benzoylperoxide (100 mg.) was added every hour for 5 hours and the reaction mixture was left at reflux for 18 hours. The mixture was chilled and filtered. The solid was washed with warm water followed by ethanol and ether. It was crystallized from THF, yield, 28-30 g. (83-85%), m.p. 205-207°; nmr (deuteriotrifluoroacetic

acid): δ 1.05 (s, 9, *t*-butyl), 4.18 (s, 2, CH_2), 7.4 (d, 1, $J_{7,8}$ = 4 Hz, H-8), 7.7 (dd, 1, $J_{7,8}$ = 4 Hz, $J_{5,7}$ = 1 Hz, H-7), 7.98 (d, 1, $J_{5,7}$ = 1 Hz, H-5).

Anal. Calcd. for $C_{14}H_{16}BrN_3O_2$: C, 49.75; H, 4.78; N, 12.42. Found: C, 49.70; H, 4.48; N, 12.15.

2-Amino-6-bromomethyl-4-hydroxyquinazoline (**9**).

Compound **8** (5.3 g., 15 mmoles) was suspended in THF (100 ml.) and warmed. To this mixture was added methanolic hydrogen chloride until a clear solution resulted. To this solution water (5 ml.) was added and the reaction mixture was heated at reflux for 4 hours, during which time a precipitate had formed. The solvents were removed *in vacuo* and the residue was washed with acetone. The product was crystallized from aqueous ethanol, yield, 3.8 g. (95%), m.p. >400°; nmr (deuteriotrifluoroacetic acid): δ 4.35 (s, 2, CH_2).

Anal. Calcd. for $C_9H_8BrN_3O$: C, 42.54; H, 3.17; N, 16.53. Found: C, 42.50; H, 3.51; N, 16.37.

Diethyl 5,8-Deazafolate (**10a**).

Diethyl 4-aminobenzoyl-L-glutamate (16) (15 g., 47 mmoles) and **9** (11.7 g., 46 mmoles) were suspended in 2-ethoxyethanol (100 ml.) and heated to 80°. Purified triethylamine (17) was added slowly until the reaction mixture became alkaline and a clear solution resulted. The reaction mixture was heated at 100° until it gave negative active halogen test (18) (approximately 4 hours). Solvents were removed *in vacuo* and the residue was washed with ethyl acetate and water. The solid was dissolved in ethanol and ethanol was removed *in vacuo*. The residue was dissolved in chloroform and the solution was poured into a column packed with silica gel (60-100 mesh) in chloroform. Then the column was washed with chloroform until no compound was present in the washings. Next, the product was eluted from the column with 10% methanol in chloroform. After removing the solvents *in vacuo*, 11.8 g. of **10a** (51.5%) was obtained, m.p. 205-210°, (lit. (3) m.p. 196-198°, impure).

Anal. Calcd. for $C_{25}H_{29}N_5O_6$: C, 60.30; H, 6.20; N, 15.30. Found: C, 60.30; H, 6.16; N, 15.50.

Diethyl N^{10}-Methyl-5,8-deazafolate (**10b**).

This compound was prepared from diethyl *N*-methyl-4-aminobenzoyl-L-glutamate (19) (13.6 g., 40 mmoles) and **9** (9.6 g., 38 mmoles) and purified by the same method employed in the case of **10a**. There was obtained 6 g. (30.6%), of **10b**, m.p. 178-182° (with preliminary softening).

Anal. Calcd. for $C_{26}H_{31}N_5O_6$: C, 61.28; H, 6.13; N, 13.74. Found: C, 60.95; H, 6.28; N, 13.78.

5,8-Deazafolic Acid (**3a**).

A mixture of **10a** (2.6 g., 5.2 mmoles) in 100 ml. of 0.2*N* sodium hydroxide was stirred at ambient temperature for 24 hours. After filtration through Celite, the solution was acidified to *p*H 4.0 with 0.1 *N* hydrochloric acid. The solid was collected on a filter, washed successively with water, acetone, and ether, and then dried *in vacuo* at 100°. There was obtained 1.6 g. (67%) of product, m.p. 232° dec. (with preliminary softening), (lit. (3) m.p. 224-240° dec.).

Anal. Calcd. for $C_{21}H_{21}N_5O_6 \cdot 0.25H_2O$: C, 56.81; H, 4.88; N, 15.78. Found: C, 56.74; H, 4.93; N, 16.00.

N^{10}-Methyl-5,8-deazafolic Acid (**3b**).

This compound was prepared in an analogous manner to that employed in the case of **3a**. The product was obtained in 65% yield, m.p. 220-225° dec.

Anal. Calcd. for $C_{22}H_{23}N_5O_6 \cdot 0.75H_2O$: C, 56.75; H, 5.28; N, 15.00. Found: C, 56.54; H, 5.44; N, 15.30.

6-(4-Carbethoxyanilinomethyl)-4-hydroxy-2-trimethylacetamidoquinazoline (**11**).

A mixture of **8** (1.7 g., 5 mmoles), ethyl 4-aminobenzoate (1.5 g., 9.1 mmoles), and ethanol (30 ml.) was heated and triethylamine (1 ml.) was added dropwise. The reaction mixture was heated at reflux for 2 hours, cooled, and filtered. The solid was crystallized from THF, yield, 1.6 g. (75%), m.p. 244-247°.

Anal. Calcd. for $C_{23}H_{26}N_4O_4$: C, 65.50; H, 6.20; N, 13.25. Found: C, 65.59; H, 6.45; N, 13.34.

2-Amino-6-(4-carbethoxybenzylamino)-4-hydroxyquinazoline (**12**).

A mixture of **9** (0.80 g., 3.1 mmoles), ethyl 4-aminobenzoate (0.8 g., 4.85 mmoles), and 50 ml. of 2-ethoxyethanol was heated to 80° and triethylamine was added dropwise until a clear solution was obtained. The heating was continued for 4 hours and then the solvent was removed *in vacuo*. The residue was suspended in ethanol and filtered and the filtrate diluted further with ether. After a second filtration, the solvents were removed *in vacuo*. The solid was separated on a filter, washed with ether and water, and then dried *in vacuo* over phosphorus pentoxide. There was obtained 0.3 g. (28%) of tan solid, m.p. 300-305 dec. This compound was shown to be identical to an authentic sample (14) by tlc, mixed m.p. and nmr.

2-Amino-4-hydroxy-6-hydroxymethylquinazoline (**13**).

A 2.0 g. sample of **8** was suspended in 20 ml. of 0.5 *N* hydrochloric acid and heated under reflux until a clear solution was obtained (*ca.* 1 hour). To insure a complete reaction, the solution was filtered and then made basic with 2*N* sodium hydroxide. After 1 hour, it was neutralized to *p*H 5.4 with glacial acetic acid. The product was isolated on a filter, washed with water and acetone, and then dried *in vacuo* over phosphorus pentoxide. There was obtained 1.0 g. (77%) of **13**, which was identical to an authentic sample (15) by tlc, mixed m.p., and nmr (deuteriotrifluoroacetic acid): δ 5.6 (s, 2, CH_2).

Anal. Calcd. for $C_9H_9N_3O_2 \cdot 0.25H_2O$: C, 55.23; H, 4.89; N, 21.47. Found: C, 55.00; H, 4.86; N, 21.16.

Acknowledgment.

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