Quinazolines as Inhibitors of Dihydrofolate Reductase. 1†

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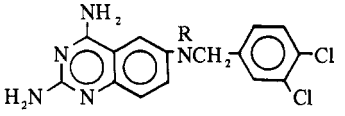
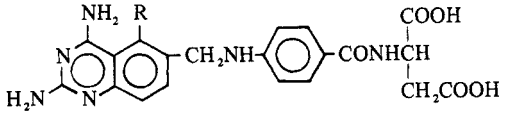
A series of 31 quinazolines bearing simple substituents was prepared and evaluated for inhibitory action against rat liver dihydrofolate reductase. Eight of these have not been reported previously, while one, 2-amino-4-mercaptoquinazoline (**22**), represents the first example of a fully aromatic quinazoline having this substitution. The 2,4-diamino derivatives were the most potent inhibitors, with the 5 position being the optimum location for activity enhancement by small nonpolar groups. The most potent compound was 6-bromo-5-chloro-2,4-diaminoquinazoline (**17**) which was nearly as potent as pyrimethamine in this assay system.

Prompted by the apparent isosterism between the quinazoline and pteridine ring systems, numerous investigators have directed their efforts toward the synthesis of quinazoline derivatives as potential chemotherapeutic agents. It has been shown that certain 2,4-diaminoquinazolines bearing simple substituents on the aromatic nucleus possess significant antibacterial effects and/or modest antimalarial action.[1-4] Reports concerning the potent antiparasitic activity of 2,4-diamino-6-(3,4-dichlorobenzylamino)quinazoline (**1a**) and its *N*-nitroso derivative **1b**[5-7] kindled a large and continuing synthetic effort on related compounds which has already yielded a number of compounds having antimalarial potencies 200–1160 times greater than quinine.[7-10] On the



1a, R = H
b, R = NO



2a, R = CH_3
b, R = Cl

other hand, classical (conjugated) folic acid analogs such as methasquin (**2a**) and chlorasquin (**2b**) have been extensively investigated and shown to have potential utility as antineoplastic agents.[11-14]

In an effort to provide a more rational basis for the design of new quinazoline antifols, a study of the inhibition of mammalian dihydrofolate reductase was initiated in this laboratory. The compounds studied, together with their physical properties and inhibitor potencies, are presented in Table I.

Most of the compounds were prepared by standard synthetic methods although there are two points which should be noted. First of all, several approaches to 2-amino-4-mercaptoquinazoline (**26**), the first member of a new series of quinazolines,‡ were unsuccessful, including the reaction of **18** with phosphorus pentasulfide and the chlorination of **25** (to be followed by reaction with potassium hydrosulfide). The desired product was finally obtained in poor yield by the reaction of **25** with phosphorus pentasulfide followed by deacetylation in dilute base. Secondly, 2-amino-4-hydroxy-5,6,7,8-tetrahydroquinazoline (**19**) and its 6-methyl derivative **21** were prepared by the acid hydrolysis of their 2,4-diamino counterparts **4** and **10**. These reactions required substantially longer reflux periods than was the case for the hydrolysis of the fully aromatic 2,4-diaminoquinazolines.

As shown in Table I, the inhibition of rat liver dihydrofolate reductase by 2,4-diaminoquinazoline (**3**) was improved only slightly, if at all, by substitution in the 6 position with small polar or semipolar groups such as NH_2 (**5**), CN (**6**), CH_2NH_2 (**7**) (essentially 100% protonated under the assay conditions), and CHO (**8**). In contrast, placement of a small nonpolar group in the 6 position, such as CH_3 (**9**), Cl (**11**), or Br (**12**), led to a five- to tenfold increase in binding. This indicates the presence of a hydrophobic region on the enzyme surface immediately adjacent to the 6 position of the bound quinazoline.

The substitution of small nonpolar groups in the 5 position of the diaminoquinazoline produced even more striking effects. A 5-CH_3 (**13**) or 5-Cl (**14**) group increased inhibitory potency 25- and 40-fold, respectively, over the parent compound. There is, therefore, an exceptionally strong hydrophobic binding region adjacent to the 5 position. These results are similar to the findings of Rosowsky, *et al.*,[1] that for diaminoquinazolines bearing small nonpolar groups, antibacterial activity with respect to substitution position followed the order 5 > 6 > 7.

This prompted the investigation of whether hydrophobic binding could be achieved simultaneously at the 5 and 6 positions. Although the 6-NH_2 group was not hydrophobic, 5-Cl-6-NH_2 (**15**) was again 40-fold more potent than 6-NH_2 (**5**). With a chloro or bromo group in the 6 position, the addition of a 5-Cl substituent produced a 20–25-fold binding increment. Thus, the 5,6-Cl_2 (**16**) and 5-Cl-6-Br (**17**) derivatives were 100- and 200-fold, respectively, more potent than the parent compound **3**. In fact, **17** was nearly as inhibitory as pyrimethamine against the rat liver enzyme and only fivefold less potent than **1a**.

2,4-Diamino-5,6,7,8-tetrahydroquinazoline (**4**) was three times as effective an inhibitor as its fully aromatic analog **3**. The related 6-methyltetrahydroquinazoline (**10**) was at least as potent as its aromatic counterpart **9**. Although substitution of a CH_3 group at the 6 position of **4** gave less than a fourfold binding enhancement, it should be kept in mind that **10** is racemic, and there may well be a substantial difference in activity between the two stereoisomers.

The results obtained above are consistent with an argument proposed by Baker[16] that the hydrophobic binding region of dihydrofolate reductase lies adjacent to the active site rather than within it. In studies on pigeon liver dihydrofolate reductase, Baker noted the hydrophobic binding contribution of the benzo moiety of 2,4-diamino-6-methylquin-

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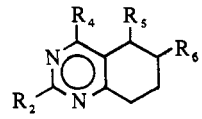
‡However, it should be noted that 2-amino-4-mercapto-5,6,7,8-tetrahydroquinazoline was previously reported.[15]

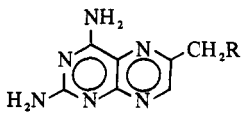
Table I. Compounds Assayed as Inhibitors of Rat Liver Dihydrofolate Reductase



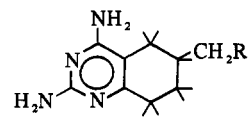
No.	R_2	R_4	R_5	R_6	Benzo ring[a]	Mp, °C	Yield (purified), %	Recrystn medium[b]	Formula[c]	I_{50},[d] μM
3	NH_2	NH_2	H	H	Ar	258–260[e]				22
4	NH_2	NH_2	H	H	H_4	242–244[f]				7.4
5	NH_2	NH_2	H	NH_2	Ar	255–257[g]				27
6	NH_2	NH_2	H	CN	Ar	380–383 dec[h]	79[i]			12
7	NH_2	NH_2	H	CH_2NH_2	Ar	235–239[j]				9.4
8	NH_2	NH_2	H	CHO	Ar	<400[h]				10
9	NH_2	NH_2	H	CH_3	Ar	248–253[k]				2.2
10	NH_2	NH_2	H	CH_3	H_4	230–232[l]				2.0
11	NH_2	NH_2	H	Cl	Ar	270–272[m]	87	A		4.0
12	NH_2	NH_2	H	Br	Ar	260–265	39	B	$C_8H_7BrN_4$	2.5
13	NH_2	NH_2	CH_3	H	Ar	212–214[n]				0.82
14	NH_2	NH_2	Cl	H	Ar	180–183[o]				0.56
15	NH_2	NH_2	Cl	NH_2	Ar	199–205[p]				0.66
16	NH_2	NH_2	Cl	Cl	Ar	251–253	60	A	$C_8H_6Cl_2N_4$	0.20
17	NH_2	NH_2	Cl	Br	Ar	275–277 dec	15	B	$C_8H_6BrClN_4$	0.10
18	NH_2	OH	H	H	Ar	315 dec[q]				49
19	NH_2	OH	H	H	H_4	303–305[r]	50	C	$C_8H_{11}N_3O$	110
20	NH_2	OH	H	CH_3	Ar	439–440 dec[s]	80		$C_9H_9N_3O$	36
21	NH_2	OH	H	CH_3	H_4	>280 dec	73	D	$C_9H_{13}N_3O$	55
22	NH_2	OH	CH_3	H	Ar	297–300	70	D	$C_9H_9N_3O$	16
23	NH_2	OH	Cl	H	Ar	321–325 dec	66	D	$C_8H_6ClN_3O$	22
24	NH_2	OH	H	(7)CF_3	Ar	339–342[t]	60	A	$C_9H_6F_3N_3O$	45
25	AcNH	OH	H	H	Ar	275–278[u]				52
26	NH_2	SH	H	H	Ar	>260 dec	9.5	E	$C_8H_7N_3S \cdot H_3PO_4$	43
27	OH	NH_2	H	H	Ar	410–412[v]				96
28	SH	NH_2	H	H	Ar	295–297 dec[w]				190
29	OH	OH	H	H	Ar	355–357[x]				130[y]
30	H	NH_2	H	H	Ar	266–269[z]				820
31	H	OH	H	H	Ar					240
32	H	SH	H	H	Ar	317.5 dec[aa]				>100[bb]
33	H	H	H	H	Ar					5500
Ia[cc]										0.018
Pyrimethamine[cc]										0.07[dd]

[a] Ar = aromatic; H_4 = 5,6,7,8-tetrahydro. [b] A = 2-methoxyethanol–H_2O (NH_4OH); B = DMF–H_2O (NH_4OH); C = DMF; D = DMF–H_2O; E = DMF–H_2O (H_3PO_4). [c] Compounds for which formulas are given were analyzed for C, H, and N; the values were within ±0.4% of theoretical values. [d] Assayed spectrophotometrically (340 mμ) with 9 μM dihydrofolate, 30 μM NADPH, and 0.15 M KCl in 0.05 M Tris buffer (pH 7.4). [e] A. Kötz [*J. Prakt. Chem.*, **47**, 303 (1893)] reported mp 248–250°. [f] E. J. Modest, S. Chatterjee, and H. Kangur [*J. Org. Chem.*, **30**, 1839 (1965)] reported mp 243–245°. [g] Davoll and Johnson[11] reported mp 255–258°. [h] Davoll and Johnson[11] reported mp <360°. [i] From **5** by Sandmeyer reaction using CuCl–KCN (**5**:CuCl:KCN, 1:2:8); a modification of the procedure of Davoll and Johnson,[11] who reported a 65% yield with $CuSO_4$–KCN. [j] Elslager, *et al.*,[8] reported mp 239–242°. [k] Rosowsky, *et al.*,[1] reported mp 255.5–256.5°. [l] E. J. Modest, S. Chatterjee, and H. Kangur [*J. Org. Chem.*, **27**, 2708 (1962)] reported mp 232–234°. [m] Rosowsky, *et al.*,[1] reported mp 269–271.5°. [n] Rosowsky, *et al.*,[1] reported mp 210–211°. [o] Davoll and Johnson[11] reported mp 183–185°. [p] Davoll and Johnson[11] reported mp 200–203°. [q] R. B. Trattner, G. B. Elion, G. H. Hitchings, and D. M. Sharefkin [*J. Org. Chem.*, **29**, 2674 (1964)] reported mp 312° dec. [r] P. C. Mitter and A. Bhattacharya [*Quart. J. Indian Chem. Soc.*, **4**, 149 (1927)] reported mp >300°. [s] V. Oakes, H. N. Rydon, and K. Undheim [*J. Chem. Soc.*, 4678 (1962)] reported mp >360°. [t] Partial melting point followed by resolidification; second mp 364–370° followed by resolidification; final mp 420–428° dec. [u] Grout and Partridge[22] reported mp 277–280°. [v] Compound softened and then resolidified at 355–365°; K. W. Breukink and P. E. Verkade [*Recl. Trav. Chim. Pays-Bas*, **79**, 443 (1960)] reported mp >350°. [w] N. K. Ralhan and H. S. Sachdev [*J. Sci. Ind. Res., Sect. B*, **19**, 215 (1960)] reported mp 300°. [x] G. Doleschall and K. Lempert [*Monatsh. Chem.*, **95**, 1068 (1964)] reported mp 351–352°. [y] By extrapolation due to insufficient solubility. [z] A. K. Macbeth and H. J. Rodda [*Nature (London)*, **156**, 207 (1945)] reported mp 272°. [aa] D. J. Fry, J. D. Kendall, and A. J. Morgan [*J. Chem. Soc.*, 5062 (1960)] reported mp 320–322°. [bb] Insufficient solubility for determination. [cc] Sample kindly provided by Division of Medicinal Chemistry, Walter Reed Army Institute of Research. [dd] Ferone, *et al.*,[17] reported 0.7 μM for I_{50} of pyrimethamine using 50 μM dihydrofolate.

azoline (**9**), which was a 22-fold better inhibitor than the corresponding pteridine **34**.[16] In contrast, a comparison of two classical inhibitors, which presumably bind to the enzyme in the same manner as folic acid, yielded opposite results, as the racemic tetrahydroquinazoline analog **36** was less than one-hundredth as potent as aminopterin **35**.[16] The tetrahydrobenzo moiety of **36** not only fails to contribute hydrophobic binding but in fact is detrimental, possibly due to its lack of planarity. The corresponding 6-methyltetrahydroquinazoline (**10**) was not available at the time of Baker's investigation, but we have now shown that racemic **10** achieves a level of hydrophobic binding similar to **9**, at least in the case of the rat liver enzyme. Therefore, it is unlikely that the methyltetrahydrobenzo moiety of **10** resides on the same enzymic site as the corresponding group of **36**. More likely, **10** and related quinazolines which lack the terminal glutamate residue are bound at the active site by the diaminopyrimido moiety but can rotate on the enzyme surface to take advantage of an adjacent hydrophobic area.[16]



34, R = H
35, R = $NHC_6H_4CONHCHCO_2H$ with $(CH_2)_2CO_2H$



10, R = H
36, R = $NHC_6H_4CONHCHCO_2H$ with $(CH_2)_2CO_2H$

In the case of pyrimidines and pteridines, the 2-amino-4-

hydroxy derivatives are normally two or three orders of magnitude poorer inhibitors of dihydrofolate reductase than the corresponding 2,4-diamino compounds.[16] Several 2-amino-4-hydroxyquinazolines were synthesized to investigate this relationship. Surprisingly, 2-amino-4-hydroxyquinazoline **(18)**, as well as 2-amino-4-mercaptoquinazoline **(26)**, was about half as effective as its diamino analog **3** in inhibiting the rat liver enzyme. However, the substitution of **18** with 6-CH_3 **(20)**, 5-CH_3 **(22)**, or 5-Cl **(23)** produced at most a threefold enhancement of activity. The relatively minor hydrophobic binding increments achieved with these substituents suggest that the spatial orientation of the 2-amino-4-hydroxyquinazolines when bound to the enzyme may not be identical with that of the diaminoquinazolines. The substitution of **18** with a 7-CF_3 group **(24)** had no effect. Although no 7-substituted diaminoquinazolines were examined in this series, Davoll, *et al.*, noted that the 7 position isomer of **1a** was devoid of antimalarial activity at a reasonably high dose level.[7] Two 2-amino-4-hydroxy-5,6,7,8-tetrahydroquinazolines **(19** and **21)** were somewhat less potent than their aromatic counterparts **18** and **20**.

Apparently the requirement of an amino group at the 2 position is more stringent than at 4, because replacement of the 2-NH_2 of **3** by OH **(27)** or SH **(28)** led, respectively, to approximately fourfold or ninefold losses in binding. Somewhat surprisingly, acetylation of the 2-NH_2 group of **18** to give **25** produced no significant change in inhibition. 2,4-Dihydroxyquinazoline **(29)** was sixfold less effective than **3**. Quinazolines with only a hydrogen in the 2 position were all poor inhibitors, regardless of whether the 4 substituent was NH_2 **(30)**, OH **(31)**, or SH **(32)**. Even quinazoline **(33)** itself showed inhibition at sufficiently high concentration.

Inhibition profiles with respect to dihydrofolate reductase have revealed substantial species differences for a few diamino heterocycles.[16,17] Thus, the results obtained with mammalian dihydrofolate reductase cannot necessarily be extrapolated to other enzyme sources. Nevertheless, it is clear that 2,4-diaminoquinazolines, appropriately substituted in the 5 and 6 positions, offer great promise for exploitation of the hydrophobic binding region. This has already been achieved with compounds like **1a**, which bears a large hydrophobic group in the 6 position. Based on this mammalian enzyme inhibition study, reasonable possibilities for activity enhancement include placement of the large nonpolar group in the 5 position rather than in 6 or simultaneous substitution of a small hydrophobic group (Cl, CH_3) at 5 and a large one at 6. In this vein, it is noteworthy that the introduction of a 5-Cl or 5-CH_3 group in an active antimalarial quinazoline can often, but not always, cause a dramatic increase in antiparasitic activity.[7,§] Also, the finding that in some cases other groups such as OH or SH can replace NH_2, especially at the 4 position without great loss in binding, deserves further study in quinazolines bearing large hydrophobic substituents. Such modifications are currently under investigation in this and other laboratories.

Experimental Section

The enzyme inhibition procedures employed are essentially the same as those described earlier.[18,19] Melting points were determined with a Fisher-Johns or a Mel-Temp apparatus and are uncorrected. All compounds had ir spectra in accord with their assigned structures and were homogeneous or nearly so on tlc (Gelman SAF). Compounds **31** and **33** were obtained commercially.

2,4-Diamino-6-haloquinazolines (11, 12, 16, 17). General Procedure. The 2,4-diamino-6-haloquinazolines were obtained from 2,4,6-triaminoquinazoline **(5)**,[11] or 5-chloro-2,4,6-triaminoquinazoline **(15)**,[11] by diazotization of the 6-amino group and treatment with the appropriate cuprous halide. The synthesis of 6-chloro-2,4-diaminoquinazoline **(11)** is typical.

A cold solution of 0.61 g (0.0035 mol) of **5**[11] in 7 ml of 2 *N* HCl was treated with a cold solution of 0.26 g (0.0038 mol) of $NaNO_2$ in 1.5 ml of H_2O. The resulting solution of the diazonium salt was swirled in an ice bath for 2 min and then added gradually to a cold, vigorously stirred solution of 0.71 g (0.007 mol) of CuCl in 5 ml of concentrated HCl. The foaming mixture was diluted with ice–H_2O when it became too thick to stir. Stirring was continued in an ice bath for 10 min, during which time the color went from brown to light green, and then at room temperature for 1 hr. After basification with concentrated NH_4OH, the mixture was stirred for several minutes at room temperature and filtered. The solid was washed with H_2O and recrystallized from 2-methoxyethanol–H_2O containing excess concentrated NH_4OH to give 0.59 g (87%) of **11** as golden crystals, mp 270–272° with slight preliminary softening (tlc in 1:2 DMF-EtOAc) (lit.[1] mp 269–271.5° for preparation by another route).

To prepare the 6-bromoquinazolines **12** and **17**, the diazotization was carried out in 2 *N* $MeSO_3H$. Addition of the diazonium salt to the CuBr, dissolved in 50% HBr, resulted in little or no reaction until the temperature was raised to *ca.* 50°. The temperature was maintained at 50–75° until N_2 evolution and decolorization were complete. (Decolorization was sometimes evident only after dilution with H_2O.) The work-up of **17** in particular was complicated by contamination of the product with Cu salts; an extraction with boiling DMF was performed prior to recrystallization.

2-Amino-4-hydroxyquinazolines (18–23). Each of these compounds was prepared from the corresponding 2,4-diaminoquinazoline by hydrolysis in refluxing 2 *N* HCl for 2–3 hr **(18, 20, 22,** and **23)**, 31 hr **(19)**, or 48 hr **(21)**. After cooling, the products were neutralized with concentrated NH_4OH, washed with H_2O, and recrystallized if necessary.

2-Amino-4-hydroxy-7-trifluoromethylquinazoline (24). A mixture of 0.465 g (0.0023 mol) of 4-trifluoromethylanthranilamide,[20] 0.32 g (0.00275 mol) of chloroformamidine hydrochloride,[21] and 1.5 ml of diglyme was heated in an oil bath. At *ca.* 130°, fairly vigorous evolution of HCl began, accompanied by a mild exotherm. Within a few minutes the reaction had subsided, and the temperature was gradually raised to the boiling point, where it was maintained for 15 min. After cooling, the solid was collected on a filter and washed with diglyme, dioxane, and EtOAc. Recrystallization with the aid of charcoal from 2-methoxyethanol–H_2O containing excess concentrated NH_4OH gave 0.31 g (60%) of nearly colorless crystals, mp 339–342° (partial), followed by resolidification and second mp 364–370°, followed again by resolidification and final mp 420–428° dec (tlc in 1:2 DMF-EtOAc). *Anal.* ($C_9H_6F_3N_3O$) C, H, N.

2-Amino-4-mercaptoquinazoline·H_3PO_4 (26). A mixture of 4.06 g (0.020 mol) of 2-acetamido-4-hydroxyquinazoline **(25)**,[22] 6.00 g of P_2S_5,# and 60 ml of C_5H_5N was stirred at 70–80° under protection from moisture and with continuous N_2 purge. After 5 hr, the mixture was cooled and added gradually to 500 ml of stirred, boiling H_2O. Stirring and boiling were continued for 2 hr. After cooling and standing, the solid was isolated by filtration and washed with H_2O. Next, the material was dissolved in 40 ml of 2 *N* NaOH and partially decolorized with charcoal. Upon standing at room temperature for 19 hr, tlc indicated that deacetylation was essentially complete. Gradual addition of excess 2 *N* HCl accompanied by scratching induced crystallization. The product was collected on a filter and washed with small volumes of 2 *N* HCl. The solid was dissolved with heating in 50 ml of H_2O, cooled, and filtered to remove some dark, insoluble material. To the filtrate was added 1 ml of 85% H_3PO_4. The crystalline salt obtained upon swirling and standing was isolated and washed with MeOH and Me_2CO. Recrystallization from DMF–H_2O containing excess H_3PO_4 yielded 0.52 g (9.5%) of light yellow crystals, mp >260° dec (tlc in 1:12 DMF-EtOAc). *Anal.* ($C_8H_7N_3S·H_3PO_4$) C, H, N.

Attempts to prepare **26** directly from 2-amino-4-hydroxyquinazoline **(18)** with P_2S_5 were unsuccessful. The insolubility of **18** necessitated a considerably higher reaction temperature, and numerous side products were obtained.

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Folic Acid Analogs. Modifications in the Benzene-Ring Region. 3. Neohomofolic and Neobishomofolic Acids. An Improved Synthesis of Folic Acid and Its Analogs†

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An improved synthesis of folic acid was developed and applied to the synthesis of neohomofolic (**13**) and neobishomofolic (**14**) acids which were desired for testing as potential anticancer agents. The procedure consists of hydrolysis of the products of sodium borohydride reduction of the anils derived from the reaction of 2-acetamido-6-formylpteridin-4(3*H*)-one (**5**) with diethyl *N*-(*p*-aminophenylacetyl)glutamate (**7**) and diethyl *N*-(*p*-aminohydrocinnamoyl)glutamate (**8**). Analogs **13** and **14** were tested on single-dose (day 1 only) and daily (qd 1–9) schedules against leukemia L1210 in mice and were inactive. Analogs **13** and **14** were not cytotoxic to cells in culture and were not inhibitory toward *Streptococcus faecium* ATCC 8043 or pigeon liver dihydrofolate reductase.

Previous efforts in these laboratories to synthesize folic acid analogs[1,2] with an altered ability to function as one-carbon transfer agents have been directed toward molecules in which the electron density at position 10 (N^{10}) is reduced. The manner in which an increase or decrease in the electron density at N^{10} in reduced folic acid analogs may potentially affect their participation in folate metabolism has been discussed previously.[1] For example, an increased electron density at N^{10} may stabilize the one-carbon transfer agents formed and render more difficult the transfer of the one-carbon unit from the tetrahydrofolate type cofactor to the substrate. We now wish to report the synthesis of *N*-[*p*-[[(2-amino-3,4-dihydro-4-oxo-6-pteridinyl)methyl]amino]-phenylacetyl]glutamic acid (**13**, neohomofolic acid) and *N*-[*p*-[[(2-amino-3,4-dihydro-4-oxo-6-pteridinyl)methyl]-amino]hydrocinnamoyl]glutamic acid (**14**, neobishomofolic acid) in which the carbonylglutamate moiety is removed from the benzene ring by one and two methylene units, respectively. Because the nonbonding electrons at N^{10} are effectively insulated from the electron-withdrawing effect of the carbonyl group, these compounds are examples of analogs in which the electron density at N^{10} is increased relative to folic acid. The added methylene units also elongate the analogs and may serve to alter their enzyme-binding abilities. It was anticipated that either or both of these effects might potentially affect folate metabolism at sites other than, or in addition to, the dihydrofolate reductase stage. Additionally, we are herein reporting an improved synthesis of folic acid and its application to the preparation of neohomofolic and neobishomofolic acids. This work is part of a program whose goal is to obtain potentially useful antineoplastic agents.

Chemistry. Neohomofolic acid (**13**) and neobishomofolic acid (**14**) were synthesized by hydrolysis of the products of $NaBH_4$ reduction of the anils **10** and **11** derived from the reactions of the pteridine aldehyde **5**[3] with **7** or **8**. The objective of preliminary efforts in the development of these procedural conditions was the model synthesis of folic acid (**12**), and we are also reporting its preparation *via* anil **9**. The synthetic procedure involved the reaction of the substituted anilines **6–8** with 1 equiv of **5** dissolved at its saturation concentration in DMSO. The anils **9–11** precipitated from this solution in high yields, and $NaBH_4$ in DMF was then used to effect reduction of the anil double bond. A ratio of 1.25 mol of $NaBH_4$ per mole of anil appeared to afford optimum yields in the model synthesis of **12** and minimized the extent of reduction of the pyrazine ring to a negligible level. Anaerobic alkaline hydrolysis simultaneously removed the glutamate ethyl esters and the N^2-acetyl blocking group and afforded the target compounds **12–14**. Purification was accomplished by utilizing DEAE-cellulose ion-exchange chromatography (Scheme I).

This improved synthesis is a modification of a procedure utilized originally by us in the synthesis[1] of 2′-azafolic acid. Subsequently, other workers reported related procedures for the preparation of pteroic acid and similar pteridines. In a synthesis of pteroic acid, Plante[4] utilized dimethyl-

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