inhibitors are binding in the same way to the same site on the enzyme. The fact that less than 12% of the variance is unaccounted for by eq 1 indicates that a variety of binding modes seems unlikely. To further explore this possibility, we factored ΣMR into two terms (MR_{R_1}, MR_{R_2}) and fit the data to this more complex equation; no improvement in correlation occurred. The data were also factored according to the type of compound: ketone, ester, amide, or aldehyde. No significant differences were found among the different classes of inhibitors. These results tend to reinforce the view that, in general, only one mode of binding on a single site is occurring.

Equation 2 constitutes another example of the reliability of correlation equations in structure–activity studies.

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Quinazolines as Inhibitors of Dihydrofolate Reductase. 4. Classical Analogues of Folic and Isofolic Acids[1a,b]

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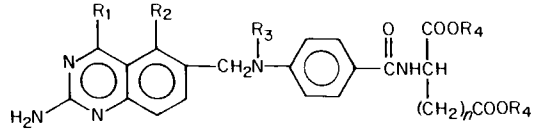
A series of classical quinazoline analogues of folic and isofolic acids was evaluated for inhibitory activity against the dihydrofolate reductases from rat liver and from *Streptococcus faecium*. Included in this group were the known active antitumor agents methasquin and chlorasquin as well as methotrexate. Two new compounds, N^{10}-formyl-5,8-deazaaminopterin and N^{10}-formyl-5,8-deazafolic acid, were synthesized specifically for this study. The latter displayed modest activity against L1210 leukemia in mice.

In the initial paper in this series quinazolines bearing relatively simple substituents were evaluated as inhibitors of rat liver dihydrofolate reductase.[2] It was observed that the 2,4-diamino configuration afforded optimum inhibitory activity and that small hydrophobic groups located in positions 5 and/or 6, but in particular the former, caused substantial increases in inhibitory potency. Next, a series of potential antimalarial agents and related compounds was evaluated against the dihydrofolate reductases from rat liver as well as from *Streptococcus faecium*.[3] As expected, compounds containing amino groups at both the 2 and 4 positions were the most effective inhibitors of either enzyme. The presence of a large hydrophobic substituent (i.e., arylthio) dramatically enhanced activity with attachment at the 6 position being more favorable than at position 5. For 6-substituted compounds replacement of the 4-NH_2 by 4-OH or 4-SH resulted in only modest decreases in activity toward the mammalian enzyme but more dramatic losses in potency were observed with the bacterial enzyme.

Recently, quinazoline analogues of pteroic and isopteroic acids were prepared and evaluated as inhibitors of the dihydrofolate reductases from the aforementioned sources.[1a] Several of the 2,4-diamino modifications were found to be exceptionally potent inhibitors of either enzyme. Interestingly, the compounds studied were not found to display the high degree of species specificity which was reported for drugs such as trimethoprim.[4] This observation suggested that compounds which closely resemble folic acid even though devoid of an amino acid residue bind to dihydrofolate reductase in a similar configuration to that assumed by classical inhibitors such as methotrexate (**1c**). A quantitative structure–activity relationship was recently formulated for quinazolines causing 50% inhibition of rat liver dihydrofolate reductase.[5] The equation derived for data generated in this laboratory was successful in correlating 101 out of 104 compounds. It was of interest, therefore, to determine whether structure–activity patterns could be elucidated for classical quinazoline analogues of folic acid (**1a**) and isofolic acid (**1b**).[6] Several of these, in particular chlorasquin and methasquin, have been evaluated as inhibitors of dihydrofolate reductase from *S. faecium*[7] as well as from human leukemia cells.[8] However, these studies did not employ a sufficient variety of analogues to allow the formulation of meaningful structure–activity patterns. In addition, the diethyl ester derivatives were not evaluated. Such information was deemed of particular importance since recent studies indicate that certain of these esters have potential utility as topical antipsoriatic agents.[9]

Chemistry. The chemical structures and physical properties of the compounds prepared for this study are summarized in Table I. The diethyl aspartates and glutamates **2**, **4**, **6**, **8**, **10**, and **12** were prepared by the reductive condensation of the required amino acid derivative with the appropriately substituted 6-cyano-

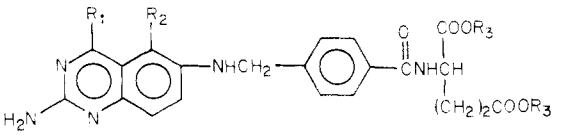
Table I. Properties of Quinazoline Analogues of Folic Acid



No.	R_1	R_2	R_3	n	R_4	Mp, °C	Method	Yield, %	Formula[a]	I_{50}, μM[b] Rat liver[c]	I_{50}, μM[b] *S. faecium*[d]
2	NH_2	H	H	1	C_2H_5	175-178[e]	A	37	$C_{24}H_{28}N_6O_5$	0.032	0.017
3	NH_2	H	H	1	H	270-280 dec[f]	B	61	$C_{20}H_{20}N_6O_5 \cdot H_2O$	0.0017	0.0062
4	NH_2	Cl	H	1	C_2H_5	194.5-196.5[g]	A	14	$C_{24}H_{27}ClN_6O_5$	0.013	0.0038
5	NH_2	Cl	H	1	H	275-290 dec[h]	B	74	$C_{20}H_{19}ClN_6O_5 \cdot 1.5H_2O$[i]	0.0064	0.0028
6	NH_2	CH_3	H	1	C_2H_5	211-213[j]	A	2		0.016	0.0057
7	NH_2	CH_3	H	1	H	275-279 dec[k]	B	97	$C_{21}H_{22}N_6O_5 \cdot 2H_2O$	0.0004	0.0025
8	NH_2	H	H	2	C_2H_5	166-169	A	27	$C_{25}H_{30}N_6O_5$	0.052	0.020
9	NH_2	H	H	2	H	>230 dec[l]	B	83	$C_{21}H_{22}N_6O_5 \cdot H_2O$	0.0034	0.0014
10	NH_2	Cl	H	2	C_2H_5	175-178[m]	A	6	$C_{25}H_{29}ClN_6O_5 \cdot 1.75H_2O$	0.031	0.0017
11	NH_2	Cl	H	2	H	228-235 dec[n]	B	85	$C_{21}H_{21}ClN_6O_5 \cdot 1.25H_2O$	0.0075	0.0007
12	NH_2	CH_3	H	2	C_2H_5	189-191[o]	A	17	$C_{26}H_{32}N_6O_5 \cdot 1.25H_2O$	0.015	0.0024
13	NH_2	CH_3	H	2	H	201-203 dec[p]	B	57	$C_{22}H_{24}N_6O_5 \cdot 1.75H_2O$	0.0069	0.0022
14	NH_2	H	CHO	2	H	211-212 dec	C	70	$C_{22}H_{22}N_6O_6 \cdot 2H_2O$	0.0015	0.0010
15	OH	H	H	2	C_2H_5					0.52	7.8
16	OH	H	H	2	H					0.023	1.6
17	OH	H	CH_3	2	C_2H_5					1.0	4.5
18	OH	H	CH_3	2	H					0.037	0.43
19	OH	H	CHO	2	H	>210 dec	C	75	$C_{22}H_{21}N_5O_7 \cdot 0.25H_2O$	0.012	0.30
1	Methotrexate									<0.001	0.0014

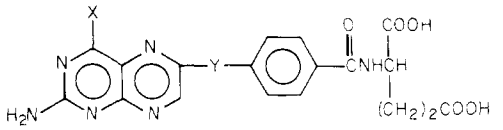
[a] Anal. C, H, N. [b] Assayed spectrophotometrically at 340 nm. [c] Conditions: dihydrofolate, 9 μM; NADPH, 30 μM; KCl, 0.15 M; in 0.05 M Tris buffer (pH 7.4); I_{50} for pyrimethamine, 0.07 μM.[3] [d] Conditions: dihydrofolate, 30 μM; NADPH, 50 μM; KPO_4, 0.05 M (pH 6.5); I_{50} for pyrimethamine, 3.0 μM.[17] [e] Davoll and Johnson[10] reported mp 174-177 °C. [f] Davoll and Johnson[10] reported mp 269-271 °C for the anhydrous material. [g] Davoll and Johnson[10] reported mp 198-200 °C. [h] Davoll and Johnson[10] reported mp 250-300 °C for the dihydrate. [i] H: calcd, 4.56; found, 5.17. [j] Davoll and Johnson[10] reported mp 211-212 °C. [k] Davoll and Johnson,[10] isolated as the disodium salt. [l] Davoll and Johnson[10] reported mp 241-242 °C for the anhydrous material. [m] Davoll and Johnson[10] reported mp 173-174 °C for the monohydrate. [n] Davoll and Johnson[10] reported mp 220-222 °C for the hemihydrate. [o] Davoll and Johnson[10] reported mp 189-191 °C for the hemihydrate. [p] Davoll and Johnson,[10] isolated as the disodium salt.

Table II. Inhibition of Dihydrofolate Reductase by Quinazoline Analogues of Isofolic Acid



				I_{50}, μM[a]	
No.	R_1	R_2	R_3	Rat liver	*S. faecium*
20	NH_2	H	C_2H_5	0.029	0.032
21	NH_2	H	H	0.026	0.032
22	NH_2	CH_3	C_2H_5	0.020	0.0039
23	NH_2	CH_3	H	0.0056	0.0083
24	OH	H	C_2H_5	0.57	24
25	OH	H	H	0.21	22
26	OH	CH_3	C_2H_5	2.7	115
27	OH	CH_3	H	0.39	5.9

[a] Cf. footnotes *b* and *c*, Table I.



1a, X = OH; Y = CH_2NH
b, X = OH; Y = $NHCH_2$
c, X = NH_2; Y = $CH_2N(CH_3)$

quinazoline according to the method of Davoll and Johnson.[10] Of these, only 8 had not been fully characterized in the earlier work. The free amino acid derivatives **3**, **5**, **7**, **9**, **11**, and **13** were obtained by saponification of the corresponding esters in dilute base. For the sake of uniformity in the enzyme assays, each compound was isolated as the free acid rather than as the disodium salt as was the case for **7** and **13**.[10] The divergence in the degrees of hydration observed in this work as compared to results obtained earlier is not unexpected for compounds of this type. The 4-OH modifications (**15**–**18**) were available for this study by virtue of synthetic work recently emanating from this laboratory.[11] Direct formylation of compounds **9** and **16** with 98% formic acid at 90 ± 5 °C yielded the N^{10}-formyl modifications **14** and **19**, respectively. The latter compound was of particular interest since its pteridine counterpart, N^{10}-formylfolate, was reported to be the most potent naturally occurring inhibitor of mammalian dihydrofolate reductase.[12] Surprisingly, the analogous reaction with the aspartate **3** was not successful. Even at 50 °C, the NMR spectrum of the product indicated that partial cleavage of the amide linkage had occurred. At ambient temperature, on the other hand, only a very low conversion occurred as adjudged by TLC. The isofolic acid analogues were available from an earlier synthetic study.[13] The inhibition of the rat liver enzyme by the latter compounds was reported earlier, but the data are included in Table II for purposes of comparison with results obtained with the bacterial enzyme.

Biological Results. Each of 26 quinazoline derivatives containing a terminal amino acid residue was evaluated for inhibition of the dihydrofolate reductases from rat liver as well as from *S. faecium*. The results, expressed as concentrations required to produce 50% inhibition of the enzymatic reaction, are presented in Tables I and II.

With respect to the 2,4-diaminoquinazoline analogues of folic acid, the diethyl esters **2**, **4**, **6**, **8**, **10**, and **12** are moderately effective inhibitors of the rat liver enzyme having I_{50}'s differing from one another by a factor of only 4.[14] Interestingly, these compounds have similar or slightly lower potency than the analogous compounds having an ethoxy group in place of the amino acid residue.[1a] The free amino acids **3**, **5**, **7**, **9**, **11**, and **13** are from 2 to 40 times more inhibitory than their respective diethyl esters with methasquin (**7**) being the most potent inhibitor of the mammalian enzyme. This correlates well with the published toxicity data, since **7** had a lower LD_{50} (mice) than methoxtrexate which in turn was more toxic than chlorasquin (**5**).[15] In general, the aspartic acid derivatives are more potent inhibitors than the corresponding glutamates, particularly in the case of **7** which is 17 times more inhibitory than **13**. It will be noted that no specific value is given for methotrexate against the rat liver enzyme. Since it has been shown that the inhibition by **1c** under these conditions is stoichiometric,[16] such a value would be meaningless. It may be argued that certain of the more potent quinazolines also fall into this category with respect to the rat liver enzyme. However, we have chosen to employ the same experimental conditions that were used in earlier work in order to permit meaningful structure–activity comparisons.

Table III. Compounds Tested against L1210 Leukemia in Mice

Compd	Dose, mg/kg (day administered)	% increase in survival[a]
14	75 (1)	0 (2T)
19	100 (1, 5)	25
	100 (1, 3)	19
	200 (1, 3)	15

[a] Testing was conducted under the direction of Dr. Glen R. Gale, Veteran's Administration Hospital, Charleston, S.C., using 10^5 rather than 10^5 cells for inoculum.[18] Controls died in an average of 6.5 days. Compounds were administered on the day(s) indicated and were dissolved in Me_2SO. Each test group consisted of six animals. Deaths occurring prior to controls are presumably due to drug toxicity and are designated T.

In the case of the bacterial enzyme it will be seen that certain of the diethyl esters of 2,4-diaminoquinazolines such as **4**, **10**, and **12** are nearly as effective as the corresponding free amino acids. These in turn are in many instances not any more effective than corresponding 5,8-deazapteroates.[1a] It is tempting, therefore, to conclude that an amino acid residue does not contribute significantly to the binding of these compounds to the *S. faecium* enzyme. The presence of a 5-Cl group affords the most potent inhibitors of the bacterial enzyme. Similar results were obtained in the case of quinazolines bearing less complex substituents.[1a] In accord with earlier studies, the replacement of a 4-NH_2 by a 4-OH group causes a much more significant reduction in potency with this enzyme than with the mammalian enzyme.[1a,3]

The introduction of a formyl group at position 10, affording the new compounds N^{10}-formyl-5,8-deazaaminopterin (**14**) and N^{10}-formyl-5,8-deazafolic acid (**19**), causes a modest enhancement of activity with respect to either enzyme. The results suggest that the general trend in potency against the bacterial enzyme for N^{10} substituents is CHO > CH_3 > H.

With one exception, the quinazoline analogues of isofolic acid, cf. Table II, are less inhibitory than their isomers having the normal folic acid configuration. However, the 5-CH_3 derivative **23** is actually somewhat more effective than **13** against the rat liver enzyme. Another surprising result is that the diethyl ester, **22**, is a twofold better inhibitor of the bacterial enzyme than its hydrolysis product **23**.

The 10-formyl modifications **14** and **19** were tested for activity against L1210 leukemia in mice and the results

are presented in Table III. Compound **14** displayed no activity and some evidence of toxicity at the 75 mg/kg dose level. This result is noteworthy since the parent compound, 5,8-deazaaminopterin (**9**), was shown to be toxic to mice at very low dosage levels.[15] This implies that enzymatic deformylation of **9** does not occur appreciably under these test conditions. Compound **19**, on the other hand, showed modest activity using three different regimens. Since these results were obtained using ten times more tumor cells in the inoculum than in the protocol employed by the National Cancer Institute, additional testing of **19** appears warranted.

Experimental Section

All analytical samples were dried in vacuo at 100 °C (P_2O_5) and gave combustion values for C, H, and N within ±0.4% of the theoretical values. Melting points were determined with a Mel-Temp apparatus and are uncorrected. All target compounds were free of significant impurities on TLC (Gelman SAF). NMR spectra were determined with a Varian T-60 spectrometer and the chemical shifts deemed critical to structural assignments are presented in parts per million (δ) downfield from Me_4Si as an internal standard. Diethyl 4-aminobenzoyl-L-glutamate was prepared according to the literature method[19] while diethyl 4-aminobenzoyl-L-aspartate was obtained according to the procedure of Davoll and Johnson.[10]

Method A (2, 4, 6, 8, 10, and 12). Each of these diethyl esters was prepared by the reductive condensation of the appropriately substituted 6-cyanoquinazoline with diethyl 4-aminobenzoyl-L-glutamate or diethyl 4-aminobenzoyl-L-aspartate in the presence of Raney nickel as originally described by Davoll and Johnson.[10] However, in the case of **10** and **12** final purification was achieved by column chromatography on silica gel using benzene–MeOH as the eluent. Compound **8**, which was not purified in the earlier work, was obtained in the following manner. The hydrogenation solvent (70% HOAc) was removed in vacuo and the remaining syrup was dissolved in EtOAc. The solution was extracted with 5% Na_2CO_3, washed with H_2O, dried over $MgSO_4$, and then concentrated in vacuo. The resulting solid was suspended in benzene and isolated by filtration.

Method B (3, 5, 7, 9, 11, and 13). In each case the requisite diethyl ester was dissolved in EtOH and treated with excess 0.1 N NaOH. When the reaction was complete as adjudged by TLC, the solution was filtered (if necessary) and then neutralized to pH 4 with 0.5 N HCl. The precipitate was separated by filtration and washed with H_2O.

Method C (14 and 19). A mixture of 0.50 g (1.13 mmol) of 5,8-deazafolic acid (**16**)[11] and 10 mL of 98% formic acid was heated at 90 ± 5 °C for 1 h. The volatile material was removed in vacuo and the residue neutralized to pH 8.5 with 1 N NH_4OH. After filtration, the solution was acidified to pH 4 with 0.5 N HCl and the resulting precipitate was separated on a filter and washed repeatedly with H_2O. After drying in vacuo at 100 °C (P_2O_5), there was obtained 0.40 g (75%) of **19** as a light tan powder. The NMR spectrum (CF_3COOD) was consistent with the assigned structure: δ 8.37 (s, 1, NCHO). Compound **14** was prepared in similar fashion and had an NMR spectrum (CF_3COOD) which was virtually identical with that of **19**.

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Hydantoins as Antitumor Agents

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A series of 27 hydantoins was prepared and tested as antitumor agents. These were variously substituted in the 5 position but with special emphasis on the substituents (chloro, acetyl, chloroacetyl, and methyl) in the 1 and/or 3 positions. The most active compound was 5,5-bis(4-chlorophenyl)-1,3-dichlorohydantoin with a T/C value of 190% against P-388 lymphocytic leukemia in mice.

This paper reports the synthesis and antitumor testing of a series of hydantoins, variously substituted in the 5 position but with special emphasis on substituents (chloro, acetyl, chloroacetyl, and methyl) in the 1 and/or 3 positions.

This work, in part, was prompted by the known activity of 1,3-dichloro-5,5-bis(4-chlorophenyl)hydantoin (**2**) against leukemia strain P-388.[1] Another stimulation to evaluate hydantoins further as antitumor agents was provided by the reported high concentrations of 5,5-diphenylhydantoin