like the assay at the 1-h incubation period.

Chemistry and Antitumor Evaluation of Selected Classical 2,4-Diaminoquinazoline Analogues of Folic Acid

J. B. Hynes,*[†] S. J. Harmon,[†] G. G. Floyd,[†] M. Farrington,[†] L. D. Hart,[†] G. R. Gale,[‡] W. L. Washtien,[§] S. S. Susten,[‖] and J. H. Freisheim[‖]

Department of Pharmaceutical Sciences, College of Pharmacy, Medical University of South Carolina, Charleston, South Carolina 29425, Veterans Administration Medical Center, Charleston, South Carolina 29402, Department of Pharmacology, Northwestern University Medical School, Chicago, Illinois 60611, and Department of Biological Chemistry, University of Cincinnati College of Medicine, Cincinnati, Ohio 45267. Received April 16, 1984

A series of six 2,4-diaminoquinazoline analogues of folic acid which bear close structural resemblance to methotrexate, 1a, were synthesized by unequivocal routes. Three of these have not been described previously, while complete structural characterization of the remaining compounds is presented for the first time. Each of the compounds was a potent inhibitor of dihydrofolate reductase (DHFR) from rat liver or L1210 leukemia cells having I_{50} values in a range similar to that of 1a. However, a wide divergence in inhibitory activity toward the growth of human gastrointestinal adenocarcinoma or L1210 leukemia cells in vitro was observed. Compounds having a normal folate configuration at positions 9 and 10 were more inhibitory than their isomeric reversed-bridge counterparts. The N-formyl modifications were the least active of the compounds studied. Unsubstituted or N-methyl modifications competed effectively with tritiated 1a for uptake into L1210 leukemia cells, while N-formyl modifications did not. Against an L1210 cell line resistant to 1a by virtue of altered transport and overproduction of DHFR, partial but not complete cross-resistance was observed for certain analogues. Of the three compounds selected for in vivo evaluation against L1210 leukemia in mice, two had a similar level of antitumor activity to that of 1a. The compound 5,8-dideazamethopterin, 2b, however, was slightly more active than 1a but at substantially reduced dose levels.

The folic acid antagonists methotrexate, 1a, and aminopterin, 1b, were among the earliest cancer chemotherapeutic agents to be developed on a rational basis.[1] Compound 1a remains a mainstay in the treatment of certain human neoplastic diseases, but the drug has numerous shortcomings. With the exception of a few high growth fraction cancers such as acute lymphocytic leukemia, choriocarcinoma, and Hodgkin's disease, most human tumors are only marginally responsive or completely refractory.[2] The use of high doses of 1a followed by rescue with calcium leucovorin has expanded somewhat the clinical utility of this drug.[3]

Mechanisms of tumor resistance to 1a include (i) overproduction of the target enzyme DHFR,[4] which has been linked to the amplification of DNA sequences containing the enzyme structural gene;[5] (ii) alterations in the membrane transport proteins for folates giving rise to reduced uptake of 1a;[6] (iii) production of a structurally altered DHFR having dramatically decreased binding affinity for 1a.[7-9] In addition, recent studies suggest that differences in the degree of intracellular poly-γ-glutamate formation may be a determinant of tumor cell responsiveness.[10]

Longer chain polyglutamates of natural folates and 1a appear to be selectively retained by most mammalian cell lines.[11-13] Differential synthesis of polyglutamates of 1a

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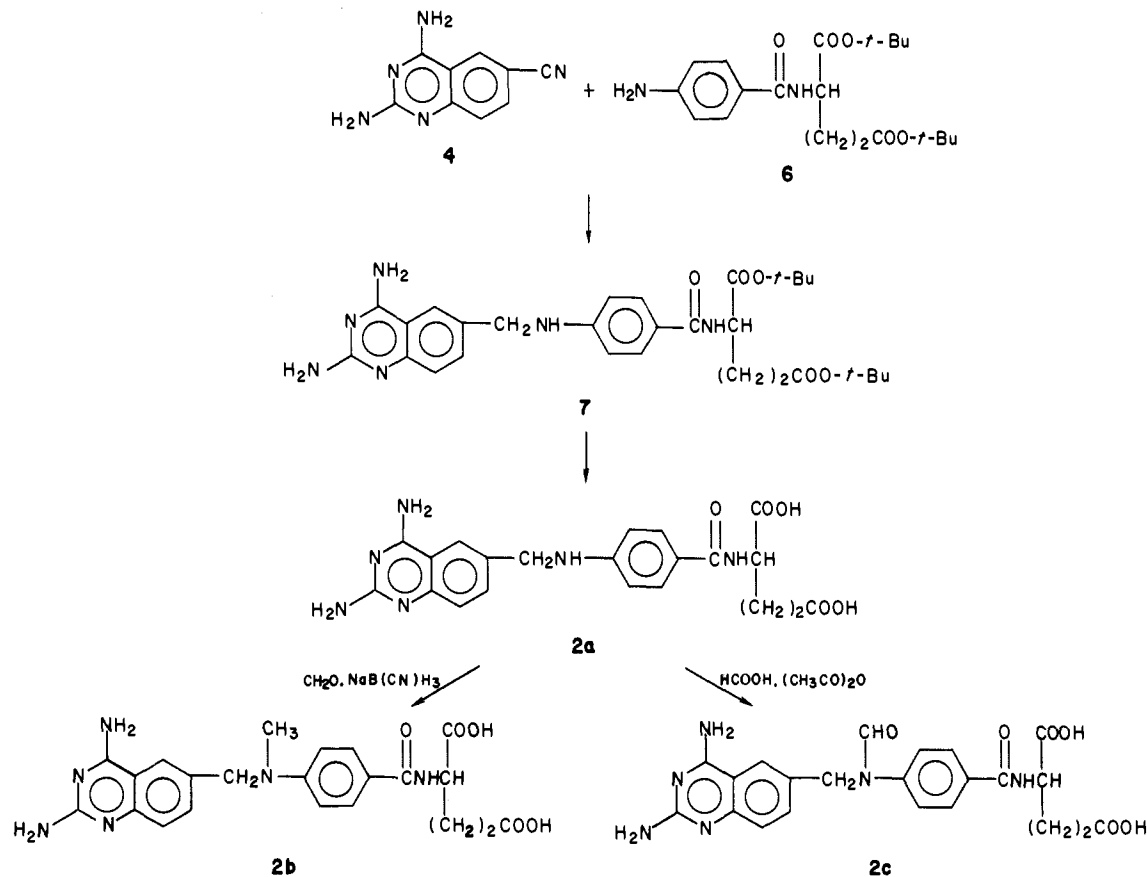
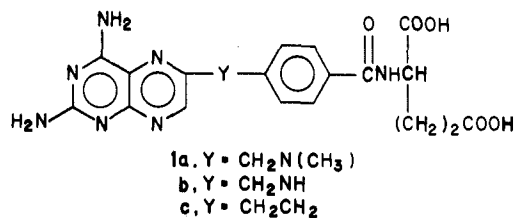†Medical University of South Carolina.
‡Veterans Administration Medical Center.
§Northwestern University Medical School.
‖University of Cincinnati College of Medicine.

Scheme I. Synthetic Route to Analogues Possessing the Normal Folate Configuration

Numerous synthetic studies have revealed that a variety of structural modifications of 1a and 1b can produce wide differences in antitumor activity as well as important pharmacokinetic parameters. For example, esters of MTX appear to act at least in part as prodrug forms which can enter cells using an alternative pathway and subsequently be hydrolyzed to yield the active cytotoxic entity. Several of the more recently synthesized esters show enhanced activity toward L1210 leukemia in mice and can result in as much as 5- to 10-fold reductions in total administered dose relative to that required for 1a.[16] In addition, the activity of the di-n-butyl ester of 1a toward tumor cells which are resistant by virtue of reduced transport of 1a indicates that compounds of this type have considerable potential in the treatment of human neoplastic disease.[17,18]

in tumor vs. normal cells may account in part for the selective toxicity of this drug.[14,15]

Subtle structural modifications at the 10-position of 1b have been found to be advantageous in enhancing selective toxcity in several murine tumor systems. This is reported to be due to more favorable uptake of drug into tumor cells vs. normal proliferative host tissue.[19,20] Of particular interest in this regard is the compound 10-deazaaminopterin, 1c, in which the 10-nitrogen is replaced by carbon.[21] A broader spectrum of effective antitumor action in mice than 1a and greater therapeutic efficacy have led to the introduction of 1c into clinical trials.[22-24] Recently, the synthesis of 8,10-dideazaaminopterin, which is structurally identical with 1c except that the 8-nitrogen is also replaced by carbon, was reported.[25] In mice bearing L1210 leukemia this compound was as effective in enhancing survival time as 1c but at one-twelfth the dose.[25]

The quinazoline counterparts of 1a and 1b can be considered as 5,8-dideaza analogues. In view of the results

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Table I. Inhibition of Growth of Human Gastrointestinal Adenocarcinoma Cells in Vitro and Rat Liver Dihydrofolate Reductase

assay	inhibition: IC_{50}, μM						
	1a	2a	2b	2c	3a	3b	3c
HuTu80	0.009	0.0035	0.007	0.12	0.15	0.015	0.36
HT29	0.018	0.0042	0.005	0.12	0.20	0.026	0.68
SW480	0.015	0.0037	0.005	0.084	0.20	0.026	0.42
WIDR	0.025	0.0038	0.008	0.18	0.20	0.039	0.98
DHFR[a]	0.0046	0.0048	0.0053	0.0049	0.0072	0.0033	0.0047

[a] Assayed spectrophotometrically at 340 nm: dihydrofolate, 9 μM; NADPH, 30 μM; KCl, 0.15 M in 0.05 M Tris buffer (pH 7.4).

obtained with other deaza derivatives of 1a and 1b, we have prepared six quinazoline analogues which differ only in the configuration of the 9,10 bridge region. Three of these have not been described previously; complete structural characterization of the remaining compounds is presented for the first time. In this report we describe preliminary in vitro biochemical and antitumor properties of these, as well as treatment experiments against L1210 leukemia in mice with the more promising analogues.

Chemistry. The synthetic approaches to the target compounds described in this report were patterned after earlier synthetic efforts.[26,27] However, modifications were introduced in order to obtain final products having a high degree of purity as adjudged by high-performance liquid chromatography (HPLC) and to preclude the possibility of racemization of the L-glutamyl moiety. The route employed for compounds having the normal folate configuration at positions 9 and 10 is outlined in Scheme I. The intermediate di-*tert*-butyl *N*-(4-aminobenzoyl)-L-glutamate, 6, was obtained by the reaction of di-*tert*-butyl L-glutamate[28] with *p*-nitrobenzoyl chloride followed by catalytic hydrogenation of the nitro group. The simplest route to 2,4-diamino-6-cyanoquinazoline, 4, was found to involve the cyclization of 2-amino-5-nitrobenzonitrile with guanidine carbonate followed by reduction with stannous chloride.[26] The conversion of the resulting 2,4,6-tri-aminoquinazoline to 4 was effected by a modification of the procedure described earlier.[26] Reductive condensation of 4 with 6 in the presence of Raney nickel yielded the diester 7, which following column chromatography on silica gel failed to give a satisfactory elemental analysis due to a high level of solvation. Deprotection of 7 using anhydrous trifluoroacetic acid afforded 5,8-dideazaaminopterin, 2a, having a high degree of purity as adjudged by HPLC. This compound was prepared earlier with use of ethyl ester protective groups, which were removed by base-catalyzed hydrolysis.[26] It has been our experience that prolonged exposure to strongly basic conditions can cause decomposition of compounds of this type and may result in racemization. Methylation of 2a using formaldehyde and sodium cyanoborohydride proceeded smoothly, yielding 5,8-dideazamethopterin, 2b, in good yield. The formylation of 2a using a mixture of formic acid and acetic anhydride at room temperature gave 10-formyl-5,8-dideazaamino-pterin, 2c. This compound was prepared earlier by heating 2a in the presence of 97% formic acid.[29] Since the 4-amino group of 2,4-diaminoquinazolines can be removed hydrolytically by heating in the presence of acids, the procedure described here is preferable. The isomeric compounds having the isofolate configuration were prepared as shown in Scheme II. The reductive condensation of di-*tert*-butyl *N*-(4-formylbenzoyl)-L-glutamate, 8,[30] with

Table II. Effects of 2,4-Diaminoquinazoline Analogues of Folic Acid upon the Uptake of Methotrexate, Growth of L1210 Leukemia Cells, and Inhibition of Dihydrofolate Reductase

no.	DHFR, I_{50},[a] μM	[³H]MTX influx, I_{50},[b] μM	growth inhib, L1210/S	IC_{50},[c] μM L1210/R81
1a	0.045	1.0	0.012	205
2a	0.048	2.1	0.021	46
2b	0.051	2.9	0.017	23
2c	0.067	95	0.58	>101[d]
3a	0.060	1.6	1.5	42
3b	0.051	4.8	0.47	>102[e]
3c	0.070	74	6.8	>103[f]

[a] Assayed spectrophotometrically at 340 nm: dihydrofolate, 113 μM; NADPH, 150 μM; in Tris buffer (pH 7.2). DHRF concentration: 9×10^{-8} M (I_{50} for 1a × 2). [b] Concentration required for 50% inhibition of influx of 1.0 μM [³H]-1a into L1210/S cells at 37 °C for 10 min. [c] Concentration required for 50% inhibition of growth (48 h), relative to untreated controls. [d] Cell growth 74% of control at this concentration. [e] Cell growth 68% of control at this concentration. [f] Cell growth 81% at this concentration.

2,4,6-triaminoquinazoline[26] in the presence of Raney nickel gave the diester 9. This was deprotected with use of anhydrous trifluoroacetic acid to afford 5,8-dideazaiso-aminopterin, 3a. This compound was prepared earlier, commencing with the diethyl L-glutamate.[27] Methylation of 3a in a fashion analogous to that employed with 2a yielded 5,8-dideazaisoamethopterin, 3b, in good yield. Finally, 9-formyl-5,8-dideazaisoaminopterin, 3c, was obtained by the direct formylation of 3a with use of formic acid and acetic anhydride.

Biological Evaluation. Each of the target compounds was evaluated as an inhibitor of DHFR from rat liver, and the results are presented in Table I. The conditions employed were the same as those used in earlier studies in order to permit comparison to values obtained with structurally related compounds.[29] It will be noted that each compound has a similar level of inhibitory potency as 1a with 3b being slightly more effective and 3a slightly less. The values reported here are in good agreement with those reported earlier for 2a as well as its 5-chloro and 5-methyl derivatives against the rat liver enzyme.[31] Compound 3a was reported earlier to be significantly less inhibitory than related compounds and 1a, and this erroneous value is hereby corrected.[29] It is believed that the original sample may have been contaminated or could have undergone decomposition prior to assay. The compounds were also studied as inhibitors of the growth of four human gastrointestinal adenocarcinoma cell lines in vitro. It will be seen that each of these is sensitive to the effects of 1a. Compound 2a was the most potent, being some 3- to 6-fold more potent than 1a. The methylated derivative 2b was also more active than 1a but somewhat less cytotoxic than 2a. On the other hand, the 10-formyl derivative 2c was

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Scheme II. Synthetic Route to Analogues Possessing the Isofolate Configuration
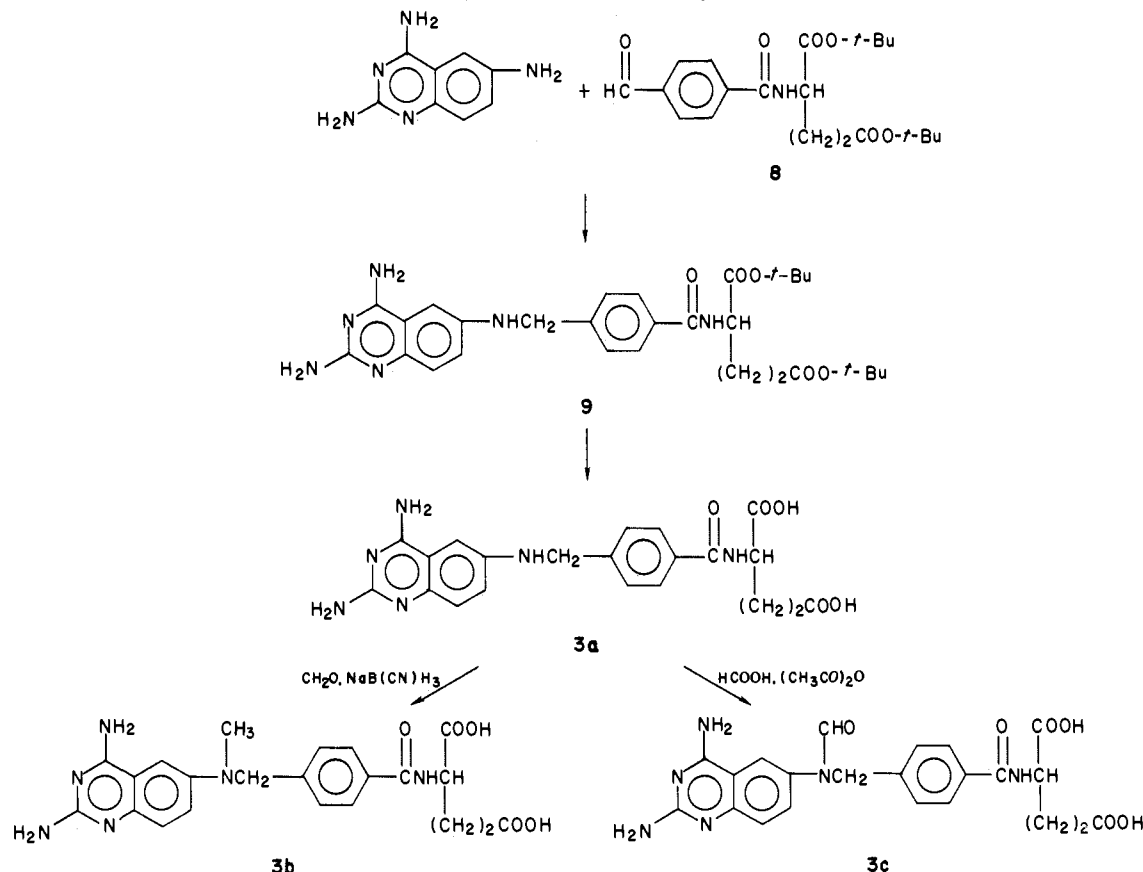
dramatically less active against all four tumor cell lines than its 10-methyl analogue or **1a**.

For compounds having the isofolate configuration, the situation was quite different with the unsubstituted derivative **3a** being approximately 1 order of magnitude less inhibitory than **1a**. The introduction of a methyl group at position 9 (**3b**) enhanced potency 5- to 10-fold, while the 9-formyl modification, **3c**, was 2- to 5-fold less active than **3a**.

The six analogues were then evaluated as inhibitors of L1210 leukemia DHFR (Table II). Each of the compounds had a similar level of inhibitory activity as that produced by **1a**. The fact that these I_{50} values are approximately 10-fold higher than those obtained with the rat liver preparation can be accounted for by the fact that greater than 10 times the concentration of DHFR was present in this assay. Nevertheless, the correlation between the results obtained using the two different enzymes is quite good. Next, the effect of these compounds upon the uptake of tritiated **1a** into sensitive L1210 leukemia cells is shown in Table II. The unsubstituted analogues **2a** and **3a** were found to be effective inhibitors of the transport of **1a**. The two methylated derivatives were somewhat less active with **2b** being more inhibitory than **3b**. Both formyl modifications, **2c** and **3c**, were dramatically less effective in preventing the influx of **1a** into these cells.

Each of the target compounds was also evaluated for inhibitory effects upon the growth of L1210 cells both sensitive (L1210/S) and resistant (L1210/R81) to **1a**. The latter cell line has been shown to have a 35-fold elevation of DHFR as well as reduced transport for **1a**.[32] Against the L1210/S cells, each of the compounds possessing a

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Table III. Antitumor Effects of Selected Compounds against L1210 Leukemia in Mice

compd (dose, mg/kg)[a]	mean survival time,[b] days	increased life span, %
control	7.4	
1a (10)	16.4	122
1a (15)	17.4	135
2b (1.5)	17.1	131
2b (2.5)	20.6	178
2b (2.75)[c]	20.7[d]	188
2b (3.0)	16.7 (4T)[e]	160[f]
3b (10)	15.0	102
3b (15)	17.7	139
3b (20)[c]	19.4[d]	169
2c (5)[c]	12.3[d]	71
2c (10)[c]	16.9[d]	135

[a] Administered sc on days 1, 3, 5, 7, 9 following intraperitoneal implantation of 10^6 cells in BDF_1 mice on day 0. [b] Ten animals per group. [c] Compound **1a** (10) gave an ILS of 161%. [d] Controls died in an average of 7.2 days. [e] Four animals died after three injections due to toxicity (T). [f] ILS for surviving animals.

normal bridge was significantly more potent than its bridge-reversed counterpart. Compounds **2a** and **2b** were nearly as effective as **1a**, while the formyl modification, **2c**, was nearly 50-fold less active. The L1210/R81 line required approximately 17 000 times the concentration of **1a** for 50% inhibition as did the sensitive cell line. Marked cross-resistance was observed for each of the analogues tested, with **2b** being the most potent of the group. The results obtained against L1210 leukemia in mice are summarized in Table III. The every other day times five regimen was selected for comparative purposes, since **1a** is most effective against L1210 leukemia using this protocol. It will be noted that **2c** and **3b** have similar activities as **1a**. On the other hand, **2b** gives a somewhat greater increase in survival than **1a** at 5- to 10-fold lower dose levels.

Discussion

From the results presented in Tables I and II it is apparent that each of the target compounds is a potent inhibitor of DHFR from rat liver as well as L1210 leukemia cells. For the former, the I_{50} values for **2a–c** and **3c** are indistinguishable with that of **1a**, while **3a** is somewhat less effective and **3b** slightly more effective. With respect to the murine enzyme, the data indicate that formylation at either position 10 (**2c**) or position 9 (**3c**) is somewhat detrimental to binding. It is not known if these slight differences in I_{50} values are indicative of structural differences between these two enzymes. It is apparent, however, that detailed kinetic analysis of these compounds as inhibitors of DHFRs will be required in order to differentiate them with regard to inhibitory potency.

It is of interest to compare the relative efficacies of these compounds on the growth of L1210/S cells vs. the four human gastrointestinal adenocarcinoma cell lines. With regard to the former, **1a** is the most potent closely followed by **2a** and **2b**. Compounds **3b** and **2c** are approximately 40-fold less effective, while **3a** and **3c** are still less active. However, against the human tumor cells, both **2a** and **2b** are considerably more effective than **1a**. The remaining analogues display the same order of decreasing growth inhibition as observed against the L1210/S cells (**3b** > **2c** > **3a** > **3c**). Results obtained with the L1210/R81 cell line show that there is a high degree of cross-resistance with each compound. However, the fact that **2b** is nearly 10-fold more effective than **1a** suggests that a component of its growth inhibitory activity may be due to interference with another folate requiring process than DHFR, and that it can enter cells via an alternative pathway than that utilized by **1a**.

The uptake studies in competition with isotopically labeled **1a** indicate that four of these compounds are taken up into L1210/S cells by the same active transport mechanism as **1a**. However, both of the formyl modifications (**2c** and **3c**) do not compete effectively. Compound **2c** presumably gains entry into tumor cells by an alternate pathway, since it displays good activity against L1210 leukemia in mice (Table III).

The selection of compounds and doses employed for in vivo antitumor studies were predicated upon the following considerations. Compound **2a** was eliminated because of its extraordinarily high toxicity to mice (LD_{50}, 0.08 mg/kg for five consecutive daily doses).[33] Its isomer, **3a**, on the other hand, showed a very low level of activity, since it afforded an ILS of 43% with use of a regimen of 85 mg/kg administered daily for five consecutive days, beginning on day one following tumor inoculation (data not shown). Earlier studies with **2c** indicated a similar or slightly lower single dose LD_{50} in mice (2/6 toxic deaths at 75 mg/kg).[29] The single dose LD_{50} for **1a** in mice was reported to be 92 mg/kg.[33] Compound **3c** was excluded from the in vivo studies due to its very low activity toward both human and L1210 tumor cells in vitro.

The results obtained against L1210 leukemia in mice (Table III) indicate that compounds **2c** and **3b** have similar levels of activity to **1a**. On the basis of their low effectiveness against L1210 leukemia cells in vitro, one would not have predicted such high efficacy. Furthermore, **2c** was approximately 10-fold less potent than **1a** toward the growth of all four human tumor cells studied, whereas **3b** had similar activity to **1a** against three of the four human cell lines. The in vivo antitumor effects of **2c** are particularly significant in terms of its mode of entry into cells.

Since it did not effectively inhibit the uptake of **1a** into L1210 cells, its influx may be governed by a process other than the "reduced folate–**1a**" transport system.

The situation with compound **2b** is entirely different since the in vitro data suggest that it should be equal to or more effective than **1a**. The results presented in Table III show that **2b** is considerably more toxic than **1a** and appears to possess greater antitumor efficacy. In this instance the replacement of the pyrazine ring nitrogens by carbon enhances antimetabolite potency. Similarly, 8,10-dideazaaminopterin is much more potent than 10-deazaaminopterin.[25] The enhanced lipophilic character of these deaza analogues may result in a slower rate of elimination than **1a**. It appears, therefore, that compounds such as **2b** could be more effective in a regimen employing less frequent administration. In addition, **2b** could be useful in high-dose antifolate therapy followed by leucovorin rescue. The greatly reduced dosage required might circumvent renal precipitation which can be a problem when **1a** is employed in this manner.[34] Finally, **2b** should be evaluated by using in vivo colon tumor models for potential use in the treatment of colorectal adenocarcinoma in view of its activity toward these types of tumor cells in vitro.

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. The question of water of solvation of target compounds was resolved in the following manner. Samples of **2c** and **3c** were dried in NMR tubes in a identical manner to the preparation of samples for elemental analysis. These were then dissolved in Me_2SO-d_6 and their ^1H NMR spectra determined and compared with that of solvent blank. Similar substantial augmentation of the H_2O resonance was observed for both compounds. All intermediates were free of significant impurities on TLC using silica gel media (Gelman SAF or Baker 1B2-F). Free glutamates (**2a–c** and **3a–c**) were assayed on cellulose (0.1 M phosphate buffer, pH 7.5). Target compounds were analyzed by HPLC with a Micromeritics Model 7000B liquid chromatograph containing a Partisil PSX 10/25 ODS-2 column (4.6 × 250 mm) 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 by HPLC. The elutions employed were a 10-min linear gradient (0–20% v/v) of MeCN in H_2O at pH 4.0 and a flow rate of 2 mL/min or a 15-min linear gradient (0–25% v/v) of MeCN in H_2O containing 0.007 M Et_3N and 0.017 M HOAc, pH 6.5, 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 in 0.1 M phosphate buffer, pH 7.0. The ^1H NMR spectra were determined with 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. Fast atom bombardment mass spectra (FAB/MS) were obtained on a Finnigan MAT 212 spectrometer using argon bombardment. Each target compound (**2a–c** and **3a–c**) exhibited an m/e corresponding to the protonated parent ion (M + 1). Di-*tert*-butyl L-glutamate was prepared by the reaction of L-glutamic acid and isobutylene under pressure as described earlier.[28] The free ester, which was found to be stable when stored at 4 °C, was employed in subsequent reactions as an oil rather than being converted into a salt derivative.

The methods of determination of the inhibition of growth of the four human gastrointestinal adenocarcinoma cell lines were described recently.[30] L1210 leukemia cells sensitive to **1a**

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(L1210/S) were grown in suspension culture as described previously.[32] The resistant cells (L1210/R81) were maintained in culture medium containing 10 μM 1a. Resistant cells were cultured in the absence of 1a for at least two transfer generations before use in order to eliminate bound 1a. Both cell lines have population doubling times of 12–14 h.

DHFR from L1210/S cells was purified by affinity chromatography as described earlier.[35] Initial rates were derived from the change in absorbance continuously recorded with a Cary Model 219 spectrophotometer. The assay mixture, in a total volume of 1 mL, consisted of 0.05 M Tris buffer (pH 7.2), 150 μM NADPH, and 113 μM dihydrofolate. The standard unit of enzyme activity was determined spectrophotometrically from the change in absorbance at 340 nm with a ΔA for the reaction of 12 300 M^{-1} at 22 °C. Inhibition studies were carried out by preincubating the enzyme, NADPH, and inhibitor in the assay buffer for 2 min at 22 °C and residual enzyme activity was determined after the addition of dihydrofolate. Remaining activity was expressed as percentage of the enzyme activity obtained in the absence of inhibitor.

The uptake of [^{3}H]-1a into L1210/S cells was measured in RPMI 1640 medium without folic acid following the procedures previously described.[36] Uptake at 37 °C was determined 10 min after the simultaneous addition of quinazoline and [^{3}H]-1a (the final concentration of [^{3}H]-1a being 1 μM). The I_{50} value represents the amount of compound required for 50% inhibition of [^{3}H]-1a uptake into drug-sensitive cells.

The growth inhibition studies using L1210/S or L1210/R81 were conducted in the following manner. Cells at an initial concentration of 5×10^4 cells/mL were grown in medium containing varying concentrations of inhibitor in 24-well cluster dishes incubated at 37 °C in a 5% CO_2 atmosphere. After 48 h, cells were counted with a Model Z_F Coulter counter (Coulter Electronics, Hialeah, FL). The IC_{50} is defined as the concentration required to reduce the number of cells in 48 h to 50% of the untreated control value.

Di-*tert*-butyl N-(4-Nitrobenzoyl)-L-glutamate (5). To a solution of 11.1 g (60 mmol) of 4-nitrobenzoyl chloride in 80 mL of 1,2-dichloroethane was added 6.06 g (60 mmol) of freshly distilled triethylamine in four portions. Next, 15.54 g (60 mmol) of di-*tert*-butyl L-glutamate in 70 mL of 1,2-dichloroethane was added slowly to the reaction flask with cooling in an ice bath. After 5 h the reaction mixture was filtered and the filtrate diluted with 100 mL of $CHCl_3$ and washed successively with 50 mL of H_2O, 50 mL of 10% $KHCO_3$, 50 mL of H_2O, 2×50 mL of 10% citric acid, 50 mL of H_2O, and 50 mL of saturated NaCl. After drying over $MgSO_4$, the solvent was evaporated under high vacuum, and the resulting solid was recrystallized from EtOH–H_2O and vacuum dried at 30 °C to yield 20.8 g (86%) of a white crystalline solid: mp 52.5–54.5 °C; TLC (cyclohexane:EtOAc, 4:1); NMR (CDCl₃) δ 8.23 (dd, 2, $H_3 + H_5$, $J_{meta} = 1.5$, $J_{ortho} = 8$ Hz), 7.93 (dd, 2, $H_2 + H_6$, $J_{meta} = 1.5$, $J_{ortho} = 8$ Hz), 7.46 (d, 1, NH, $J = 7.5$ Hz), 4.66–4.40 (m, 1, CH), 2.53–1.90 (m, 4, $(CH_2)_2$), 1.46 (s, 9, $C(CH_3)_3$), 1.33 (s, 9, $C(CH_3)_3$). Anal. ($C_{20}H_{28}N_2O_7$) C, H, N.

Di-*tert*-butyl N-(4-Aminobenzoyl)-L-glutamate (6). An 18.94-g (46 mmol) sample of 5 in 280 mL of EtOH containing 0.5 g of 10% Pd/C was hydrogenated at low pressure for 24 h. After filtration the EtOH was removed under vacuum and the resulting solid was dried under vacuum over P_2O_5 at room temperature. There was obtained 16.5 g (94%) of a white crystalline solid: mp 138–140 °C; TLC (cyclohexane:EtOAc, 4:1); NMR (CDCl₃) δ 7.56 (dd, 2, $H_2 + H_6$, $J_{meta} = 1.5$, $J_{ortho} = 8$ Hz), 6.73 (d, 1, NH, $J = 7.5$ Hz), 6.56 (dd, 2, $H_3 + H_5$, $J_{meta} = 1.5$, $J_{ortho} = 8$ Hz), 4.75–4.48 (m, 1, CH), 3.90 (br s, 2, NH_2), 2.46–1.75 (m, 4, $(CH_2)_2$), 1.45 (s, 9, $C(CH_3)_3$), 1.38 (s, 9, $C(CH_3)_3$). Anal. ($C_{20}H_{30}N_2O_5$) C, H, N.

5,8-Dideazaaminopterin (2a). A mixture of 3.5 g (18.92 mmol) of 2,4-diamino-6-cyanoquinazoline[26] and 7.5 g (19.87 mmol) of 6 in 200 mL of 70% acetic acid was hydrogenated in the presence of Raney Ni (500 mg) until H_2 uptake ceased. After the addition of charcoal, the catalyst was removed by filtration and the filtrate

was basified to pH 8.5 with NH_4OH. The precipitate was separated by filtration and washed with H_2O. The product was dissolved in $CHCl_3$ with insoluble particles being removed by filtration. The solution was washed several times with 5% citric acid and H_2O and then dried over $MgSO_4$. The solvent was removed under vacuum, and the product was dried at room temperature over P_2O_5 under vacuum for 4 h to yield 9.0 g of crude material. Purification by silica gel chromatography using $CHCl_3$:MeOH (90:10), followed by elution of the product with MeOH, gave a low recovery of a brown glassy solid having a poorly defined melting range. Elemental analysis indicated a high degree of solvation and this compound was employed in the succeeding step in this condition.

A 3.0-g sample of this impure di-*tert*-butyl ester (7) was dissolved in 45 mL of CF_3COOH and the resulting solution stirred for 1 h. The mixture was poured into 90 mL of cold Et_2O to precipitate the product. After filtration the solid was washed with Et_2O and H_2O. Next, it was suspended in 100 mL of H_2O and basified to pH 8.5 with NH_4OH. The product was then precipitated at pH 4 by the addition of 2 N HCl. It was collected by filtration, washed with H_2O and $(Me)_2CO$, and dried in vacuo over P_2O_5. There was obtained 1.37 g of beige solid (57% based upon an unsolvated reactant): mp >205 °C dec (lit.[26] mp 241–242 °C, anhydrous); TLC; HPLC; UV λ_{max} 228 nm (ϵ 41.5 × 10³), 292 (20.0 × 10³); NMR (CF_3COOD) δ 8.35–7.20 (m, 7, aromatic), 5.23 (m, 3, CH_2N + NCH), 3.13–2.30 (m, 4, $(CH_2)_2$). Anal. ($C_{21}H_{22}N_6$-O_5·1.5H_2O) C, H; N: calcd, 18.05; found, 17.47.

5,8-Dideazamethopterin (2b). A 610-mg (1.39 mmol) sample of 2a was dissolved in 35 mL of 0.1 N NaOH maintained in a N_2 atmosphere. Additional 1 N NaOH was added until complete dissolution occurred. The pH was adjusted to 6.3 with 1 N HCl. Then 0.55 mL (6.95 mmol) of 38% CH_2O was added followed by the stepwise addition of 175 mg (2.78 mmol) of $NaB(CN)H_3$. The pH of the reaction mixture was maintained near 6.3 by periodic addition of 1 N HCl and then left to stir overnight. The solution was basified to pH 12 with 1 N NaOH, stirred for 10 min, and then acidified to pH 4 with 1 N HCl. The precipitate was isolated by filtration, washed with H_2O, and dried in vacuo, yielding 548 mg (87%) of tan powder: mp 208–210 °C dec; TLC; HPLC (no 2a was detected in this sample); UV λ_{max} 228 nm (ϵ 44.4 × 10³), 305 (23.6 × 10³); NMR (CF_3COOD) δ 8.6–7.55 (m, 7, aromatic), 5.07 (m, 3, CH_2N + NCH), 3.62 (s, 3, NCH_3), 2.95–2.15 (m, 4, $(CH_2)_2$). Anal. ($C_{22}H_{24}N_6O_5$·2H_2O) C, H, N.

10-Formyl-5,8-dideazaaminopterin (2c). A 720-mg (1.6 mmol) sample of 2a was stirred in a mixture of 10 mL of $(Ac)_2O$ in 20 mL of HCOOH (97%) for 1.5 h. The product was precipitated by addition of excess Et_2O, separated by filtration, and washed with Et_2O and H_2O. The solid was suspended in 25 mL of H_2O, basified to pH 9 with 1 N NH_4OH, and quickly reprecipitated by addition of 0.5 N HCl to pH 4. After refrigeration, the product was isolated by filtration, washed with H_2O, $(Me)_2CO$, and Et_2O. After drying in vacuo, there was obtained 536 mg (70%): mp >210 °C dec (lit.[29] mp 211–212 °C dec); TLC; HPLC; UV λ_{max} 235 nm (ϵ 37.8 × 10³); NMR (CF_3COOD) δ 8.79 (s, 1, NCHO), 8.3–7.25 (m, 7, aromatic), 5.5–5.1 (m, 3, CH_2N + NCH), 3.19–2.30 (m, 4, $(CH_2)_2$). Anal. ($C_{22}H_{22}N_6O_6$·3H_2O) H, N; C: calcd, 50.77; found, 50.16.

Di-*tert*-butyl 5,8-Dideazaisoaminopterin (9). A mixture of 6.2 g (15.9 mmol) of di-*tert*-butyl N-(4-formylbenzoyl)-L-glutamate, 8,[30] and 2.68 g (15.3 mmol) of 2,4,6-triaminoquinazoline[26] in 200 mL of 70% CH_3COOH was hydrogenated at low pressure in the presence of 1 g of moist Raney Ni until H_2 uptake ceased. After the addition of charcoal, the solution was filtered and then neutralized to pH 8 with NH_4OH. The precipitate was separated by filtration and washed with H_2O and hexane. The ester was dissolved in 200 mL of $CHCl_3$ and washed successively with 2×100 mL of 10% $NaHSO_3$, 100 mL of H_2O, 2×100 mL of 5% citric acid, and 100 mL of H_2O. After drying over $MgSO_4$, the solvent was removed under vacuum and the solid was dried over P_2O_5 to yield 5.4 g (64%): mp 140–142 °C; TLC (CHCl₃:MeOH, 85:15). Anal. ($C_{29}H_{38}N_6O_5$·0.5H_2O) C, H, N.

5,8-Dideazaisoaminopterin (3a). A 2.27-g (4.1 mmol) sample of 9 was stirred in 28 mL of CF_3COOH for 1 h. The solution was added to 150 mL of Et_2O and the resulting precipitate was separated by filtration and washed with H_2O and Et_2O. The solid was suspended in 100 mL of H_2O and basified to pH 8.5 with

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NH$_4$OH and then reprecipitated by the addition of 2 N HCl to pH 4. The product was collected on a filter, washed with H$_2$O and (Me)$_2$CO, and dried in vacuo over P$_2$O$_5$. There was obtained 1.08 g (60%): mp >200 °C dec (lit.[27] mp 247–249 °C dec, hemihydrate); TLC; HPLC; UV λ$_{max}$ 242 nm (ε 49.1 × 10^3); NMR (CF$_3$COOD) δ 8.04–7.24 (m, 7, aromatic), 5.33–5.00 (m, 3, NCH$_2$ + NCH), 3.18–2.30 (m, 4, (CH$_2$)$_2$). Anal. (C$_{21}$H$_{22}$N$_6$O$_5$·2.5H$_2$O) C, H, N.

5,8-Dideazaisoamethopterin (3b). A 600-mg (1.37 mmol) sample of **3a** was added to 33 mL of 0.1 N NaOH with stirring in a N$_2$ atmosphere. A few drops of 1 N NaOH were added to effect complete dissolution and the pH was then adjusted to 6.3 by the addition of 1 N HCl. Next, 0.54 mL (6.85 mmol) of 38% CH$_2$O was added followed by slow addition of 172 mg (2.74 mmol) of NaB(CN)H$_3$. The pH was maintained at approximately 6.3 by periodic addition of 1 N HCl. After the addition was complete, the mixture was allowed to stir overnight. The solution was then basified to pH 12 with 1 N NaOH, stirred for 10 min to allow HCN gas to escape, and then neutralized to pH 4 with 1 N HCl. The precipitate was separated by filtration, washed with H$_2$O, and dried at 100 °C to yield 556 mg (90%) of greenish powder: mp 233–240 °C dec; TLC; HPLC (no **3a** was detected in this sample); UV λ$_{max}$ 245 nm (ε 40.6 × 10^3), 285 (14.1 × 10^3); NMR (CF$_3$COOD) δ 8.41–7.30 (m, 7, aromatic), 4.9 (m, 3, NCH$_2$ + NCH), 3.51 (s, 3, NCH$_3$), 2.8–2.1 (m, 4, (CH$_2$)$_2$). Anal. (C$_{22}$H$_{24}$N$_6$O$_5$·2.5H$_2$O) C, H, N.

10-Formyl-5,8-dideazaisoaminopterin (3c). A 480-mg (1.1 mmol) sample of **3a** was dissolved in a mixture of 5 mL of (Ac)$_2$O and 10 mL of HCOOH (97%) and stirred for 1.5 h. The solution

was added to 75 mL of Et$_2$O and refrigerated. The resulting solid was isolated by filtration and then washed with Et$_2$O and H$_2$O. The solid was suspended in 25 mL of H$_2$O and basified to pH 9 with 1 N NH$_4$OH. The pH was then adjusted to 4 with 0.5 N HCl. The precipitate was separated by filtration, washed with H$_2$O, (Me)$_2$CO, and Et$_2$O and then vacuum dried to yield 446 mg (87%): mp >321 °C dec; TLC; HPLC; UV λ$_{max}$ 235 nm (ε 44.5 × 10^3); NMR (CF$_3$COOD) δ 8.73 (s, 1, NCHO), 8.10–7.25 (m, 7, aromatic), 5.4–5.03 (m, 3, NCH$_2$ + NCH), 3.15–2.30 (m, 4, (CH$_2$)$_2$). Anal. (C$_{22}$H$_{22}$N$_6$O$_6$·3H$_2$O) C, H, N.

Resolved Monophenolic 2-Aminotetralins and 1,2,3,4,4a,5,6,10b-Octahydrobenzo[*f*]quinolines: Structural and Stereochemical Considerations for Centrally Acting Pre- and Postsynaptic Dopamine-Receptor Agonists

Håkan Wikström,*[†] Bengt Andersson,[†] Domingo Sanchez,[†] Per Lindberg,[†] Lars-Erik Arvidsson,[‡] Anette M. Johansson,[‡] J. Lars G. Nilsson,[‡] Kjell Svensson,[§] Stephan Hjorth,[§] and Arvid Carlsson[§]

Organic Chemistry Unit, Department of Pharmacology, University of Göteborg, S-400 33 Göteborg, Department of Organic Pharmaceutical Chemistry, Biomedical Center, University of Uppsala, S-75 123 Uppsala, and Department of Pharmacology, University of Göteborg, S-400 33 Göteborg, Sweden. Received March 22, 1984

A detailed structure–activity relationship is revealed for resolved, centrally acting dopamine (DA) agonists acting on both pre- and postsynaptic DA receptors. The compounds resolved are 5- and 7-hydroxy-2-(di-*n*-propylamino)tetralin and *cis*- and *trans*-7-hydroxy-4-*n*-propyl-1,2,3,4,4a,5,6,10b-octahydrobenzo[*f*]quinoline. By the superimposition of the structures of the more active enantiomers of these compounds with those of known dopaminergic agonists, apomorphine and ergolines, a new DA-receptor model is proposed as an outgrowth of current DA-receptor theories. One of the most important concepts of this receptor model is its emphasis on the possible positions taken by the N-substituents of dopaminergic compounds. One of these positions is sterically well defined while the other direction is sterically less critical. The model has been used to explain the lack of dopaminergic activity of some previously reported structures and also to predict properties of novel structures, including inherent chirality, which should be active at DA receptors. Hopefully, this heuristic DA-receptor model will lead to the discovery of more selective and potent pharmacological tools, which ultimately might lead to the development of therapeutic agents for treating diseases of dopaminergic function in the central nervous system.

In recent years much interest has focused on the stereochemical aspects of drug action.[1] In the dopaminergic field, the two well-known research tools apomorphine and LSD are both optically pure and stereochemically defined entities, i.e., apomorphine has the 6a*R* and LSD the 5*R*,8*R* configuration.

By comparison of these two molecules (Figure 1) with respect to their incorporated 2-aminotetralin structures,

it is obvious that the chiral carbon atoms have opposite stereochemistry. This has led several authors to propose that the pyrroleethylamine moiety of ergots confers dopaminergic properties to this class of compound.[2–4] All available data indicate that it is the phenethylamine, or

[†] Organic Chemistry Unit, Department of Pharmacology, University of Göteborg.
[‡] University of Uppsala.
[§] Department of Pharmacology, University of Göteborg.

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