Quinazoline Antifolates Inhibiting Thymidylate Synthase: Variation of the Amino Acid

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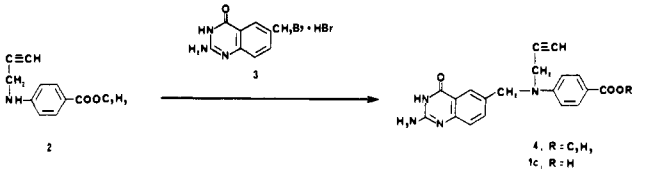
Five new analogues (**1c–g**) of the antifolate N^{10}-propargyl-5,8-dideazafolic acid (**1a**) are described in which the benzoyl-L-glutamate moiety was replaced by benzoic acid (desglutamyl-N^{10}-propargyl-5,8-dideazafolic acid), benzoyl-L-aspartate, 4-phenylbutyrate, benzoylglycine, and benzoyl-L-alanine. The esters of the appropriate 4-aminophenyl (benzoyl) starting materials were sequentially alkylated upon nitrogen, first with a propargyl halide and then with 2-amino-6-(bromomethyl)-4-hydroxyquinazoline hydrobromide. Saponification of the antifolate esters so produced gave the desired analogues. The new derivatives (**1c–g**) and also the known diethyl ester of **1a** (**1b**) were tested for their inhibition of purified L1210 thymidylate synthase (TS) and for their inhibition of the growth of L1210 cells in culture. The TS inhibition of the analogues **1b–g** was estimated by calculating the inverse relative potency, defined as the ratio IC_{50}(compound)/IC_{50}(**1a**). The results obtained were as follows: >62, 84, 9, 333, 21, and 5, respectively. All were thus less inhibitory than **1a**. None of the compounds improved upon **1a** in inhibiting the growth of L1210 cells in culture.

The effect of variation of the amino acid moiety of folate molecules on their binding to thymidylate synthase (TS, EC 2.1.1.45) has been previously studied. First, Plante and co-workers[1] compared the substrate activity of many (+)-tetrahydropteroyl amino acids with that of (+)-(tetrahydropteroyl)-L-glutamic acid ((+)-tetrahydrofolate), the latter being the precursor to 5,10-methylenetetrahydrofolate, the natural substrate for TS. The requirement of an amino acid moiety in the folate molecule followed from the absence of activity in (+)-tetrahydropteroic acid itself. Also shown to be inactive were the α-glutamylglutamic acid, β-alanine, and γ-aminobutyric acid analogues, implying that the α-carboxyl group in the acid was essential for binding. However, the D-glutamate, glycine, and D,L-alanine analogues, which incorporated this structural feature, were also inactive. In the α-amino dicarboxylic acid series, variation of the chain connecting the distal carboxyl group showed that lengthening it (D,L-α-aminoadipic acid, D,L-α-aminopimelic acid) was preferable to shortening it (L-aspartate); however, none improved upon glutamic acid. The lysine analogue was an active cosubstrate, implying that the γ-carboxylate was not essential for binding. A later report showed that (tetrahydropteroyl)polylysines containing up to five residues and carrying net positive charges were also good substrates for the *Escherichia coli* synthase.[2] Further studies of similar amino acid analogues in the tetrahydrohomopteroyl series (comparing now with tetrahydrohomofolate—a noted TS inhibitor in vitro)[1,3] gave parallel results for substrate binding except that here the lysine analogue was a poor inhibitor.[2] The foregoing study utilized TS from *E. coli* B cells. Slavik and Zakrzewski,[4] using the same enzyme, found that the diamide of (±)-5,10-methylene(tetrahydropteroyl)-D-glutamate was inactive either as substrate or inhibitor.[5] Szeto et al. studied the inhibition of human TS by several amino acid analogues of methotrexate and found that the analogue incorporating the positional isomer of glutamic acid, β-aminoglutaric acid, inhibited almost as well as methotrexate itself. From this result, and others, these authors concluded that the absolute requirement for inhibition is the presence of a free carboxyl group in the proximity of the α-carboxyl of the glutamic acid.[6]

Scheme I



This comprehensive survey indicates that replacement of the L-glutamic acid in folate molecules by other amino acids has brought no improvement in binding to TS. L-Glutamic acid is found in the quinazolinyl antifolate N^{10}-propargyl-5,8-dideazafolic acid (**1a**)[7] a new, potent, and specific inhibitor of TS.[8] Previous studies of quinazoline-based antifolates have shown tht glutamic acid is necessary for potent inhibition. However, apart from the diethyl glutamate[9] and the aspartate[10,11] analogues, no variation of the amino acid has been attempted in this series. In this paper we record the synthesis and biological activity of five new analogues (**1c–g**) of **1a** containing respectively in place of the benzoyl-L-glutamate moiety the following variants: benzoic acid (the desglutamate of **1a**), benzoyl-L-aspartate, 4-phenylbutyrate, benzoylglycine,

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Scheme II

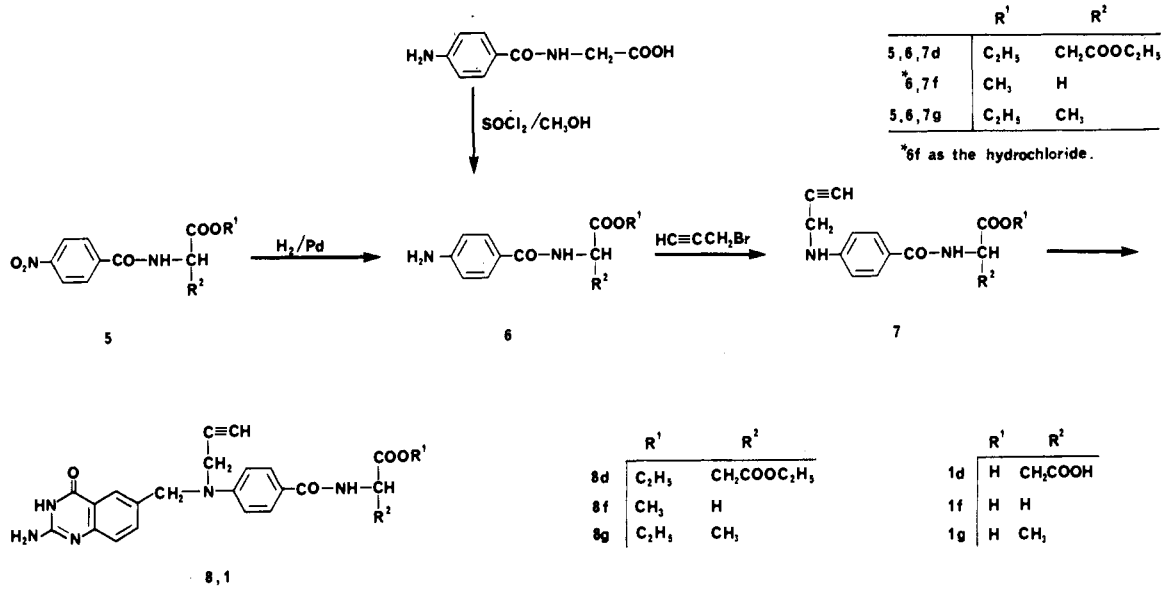
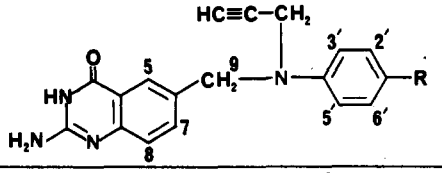


Table I. ^{1}H NMR Spectra of Antifolate Acids 1[a]



compd	spectrom freq, MHz	chemical shift, δ ≡CH (t)	propargyl CH_2 (d)	CH_9[2] (s)	NH_2 (br s)	3′,5′ (d)	2′,6′ (d)	H^8 (d)	H^7 (dd)	H^5 (d)	lactam (br)	R
1c	250	3.23	4.32	4.71	6.90	6.83	7.75	7.24	7.54	7.81	12.2	*b*
1d	90	3.12	4.28	4.68	6.40	6.88	7.72	7.18	7.52	7.82	*c*	*d*
1e	250	3.13	4.09	4.48	6.38	6.78	7.00	7.16	7.50	7.80	11.3	*e*
1f	250	3.22	4.28	4.66	6.40	6.85	7.72	7.17	7.49	7.79	11.5	*f*
1g	90[g]	3.14	4.26	4.66	6.36	6.84	7.76	7.16	7.50	7.76	*c*	*h*

[a] Spectra determined in Me_2SO-d_6. [b] 12.2 (br, 2 H, carboxyl plus lactam). [c] Not recorded. [d] 2.74 (m, 2 H, CH_2CO), 4.68 (m, 1 H, CH), 8.24 (d, J = 8 Hz, 1 H, NH), carboxyls not recorded. [e] 1.73 (quintet, 2 H, CH_2[3]), 2.19 (t, 2 H, CH_2), 2.46 (t, 2 H, CH_2), 11.3 (br, 2 H, carboxyl plus lactam). [f] 3.87 (d, J = 6.0 *Hz*, 2 H, CH_2), 8.49 (t, J = 6.0 Hz, 1 H, NH), 11.5 (br, 2 H, carboxyl plus lactam). [g] Run at 323 K. [h] 1.36 (d, J = 7 Hz, 3 H, CH_3), 4.40 (m, 1 H, CH), 8.17 (d, J = 7 Hz, 1 H, NH), carboxyl not recorded.

benzoyl-L-alanine. Four of these analogues (**1d–g**) were designed as potential inhibitors on account of containing a carboxyl group placed to mimic the α-carboxyl of L-glutamic acid. The biological activity of the known[8] diethyl ester of 1a (**1b**) is also described.

Chemistry

The desglutamate analogue **1c** was prepared according to Scheme I. *N*-Propargylbenzocaine (**2**)[12] was coupled to 2-amino-6-(bromomethyl)-4-hydroxyquinazoline hydrobromide (**3**)[13] to give the highly insoluble benzoate ester **4**. Base hydrolysis using dimethyl sulfoxide as cosolvent gave the desired acid **1c**. The aspartic acid, glycine, and alanine analogues **1d,f,g** were prepared according to Scheme II. 4-Nitrobenzoyl chloride was coupled to diethyl L-aspartate and ethyl L-alaninate to give the nitro derivatives **5d,g** which were hydrogenated to give the amines **6d,g**. 4-Aminohippuric acid was treated with thionyl chloride and methanol[14] to provide the methyl ester hydrochloride **6f**. Alkylation of the primary amines **6d,f,g**

Scheme III

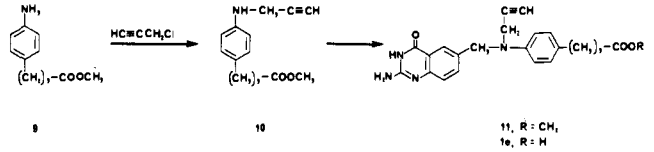


Table II. Ultraviolet Spectra of Antifolates[a]

compd	λ_{max}, nm	ε	λ_{min}, nm	ε
1c	300[b]	18 500	250.5	11 500
	279	24 800		
	228.5	43 000		
1d	301.5	26 200	285.5	23 800
	279.5	24 200	251	10 500
	228.5	50 300		
1e	325	3 900	296	2 600
	274[b]	16 000		
	267[b]	16 300		
	230.5	46 400		
1f	300	24 300	251	9 800
	281[b]	22 700		
	228	46 100		
1g	303	26 100	289.5	25 000
	280.5	25 900	253.5	12 600
	231	51 300		

[a] Spectra determined in 0.1 N aqueous sodium hydroxide. [b] Shoulder.

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Table III. Biological Properties of Antifolates

compd	acid moiety	inv rel pot.[a,b] for inhibit of L1210 thymidylate synthase	ID_{50} for L1210 cells in culture,[c] μM
1a	Glu (CB3717)	1[d]	5
1b[e]	diethyl CB3717	>62	52
1c	desglutamate	84	>200[f]
1d	aspartate	8.9	>137[g]
1e	butyrate	333	>10[h]
1f	glycine	20.7	>100[h]
1g	alanine	5.2	>10[i]

[a] For method see ref 19. [b] Defined as IC_{50}(compound)/IC_{50}(1a) determined in the same test. [c] Compounds 1c–g were not tested at higher concentrations because of their limited solubility. [d] IC_{50} = (2.00 ± 1.62) × 10^{-8} M; range (0.72–4.80) × 10^{-8} M. [e] Reference 8. [f] 11% inhibition at this concentration. [g] 25% inhibition at this concentration. [h] No inhibition at this concentration. [i] 20% inhibition of this concentration.

with propargyl bromide gave the secondary amines **7d,f,g**. Further alkylation with **3** gave the antifolate esters **8d,f,g**, which on saponification yielded the desired analogues **1d,f,g**. The 4-(aminophenyl)butyric acid analogue **1e** was prepared in similar manner according to Scheme III. However, treatment of the 4-alkylaniline **9**[15] with propargyl chloride instead of propargyl bromide was preferred in order to minimize bis-alkylation. All of the analogues **1c–g** were obtained in gelatinous form and required washing by the technique of repeated suspension–centrifugation–decantation to obtain them analytically pure. Their structures were affirmed by NMR spectroscopy (Table I) and UV spectroscopy (Table II).

Results and Discussion

The values of inverse relative potency for the inhibition of L1210 thymidylate synthase, shown in Table III, for the analogues **1c–g** are all greater than unity, indicating no improvement over **1a**. The diethyl ester **1b** was a very poor inhibitor, indicating the requirement of free carboxyl(s) for good inhibition. Removal of the glutamate residue (compound **1c**) decreased the TS inhibition by 84-fold emphasizing the importance of an amino acid residue for strong binding. The aspartic acid analogue **1d**, closest in structure to **1a**, had only about one-tenth the activity. A similar result was found in the quinazoline series for *E. coli* enzyme[10] but not for *Diplococcus pneumoniae* enzyme where comparable inhibitions were observed.[11]

Molecular models show that the carboxyl group of the butyric acid **1e** and the α-carboxyl of **1a** are equidistant from the phenyl ring. We hoped therefore that the carboxyl of **1e** was positioned to interact with the putative binding site in TS for the α-carboxyl. However, the TS inhibition shown by **1e**, over 2 orders of magnitude less than that of **1a**, did not support this contention. Restoration of the amide bond, as in the glycine analogue **1f**, improved the inhibition 16-fold (333/20.7). Rosowsky and Forsch have commented on the importance of this amide bond in another series of antifolates.[16] Without making further analogues it is difficult to tell whether the constrained geometry of the amide link helps the α-carboxyl to find an anchor point on the TS enzyme or the amide link is itself binding. Homologation of the glycine **1f** to the L-alanine **1g** increased the TS inhibition 4-fold, giving an inhibitor nearly as potent as **1a**. The methyl group is probably involved in hydrophobic bonding.[1]

All the compounds **1b–g** were less soluble than **1a**, which limited their evaluation as inhibitors of the growth of L1210 cells in culture (Table III). However, all were demonstrably less potent than **1a**.

This study has concerned the inhibition of thymidylate synthase by classical N^{10}-propargyl-5,8-dideazafolates. We draw two main conclusions: First, L-glutamic acid best promotes binding to TS. Second, the amide linkage formed from the benzoyl group and the amino acid is also necessary for strong binding.

Experimental Section

General methods and abbreviations are detailed in ref 19.

Ethyl 4-[*N*-[(2-Amino-4-hydroxy-6-quinazolinyl)methyl]prop-2-ynylamino]benzoate (4). A solution of ethyl 4-(prop-2-ynylamino)benzoate (**2**)[12] (2.03 g, 10 mmol) and **3**[13] (3.35 g, 10 mmol) in DMA (70 mL) with $CaCO_3$ (3.00 g, 30 mmol) in suspension was stirred at 25 °C for 65 h. The mixture was filtered and the filtrate concentrated to dryness on a rotary evaporator (45 °C (0.2 mm)) to give a yellow solid, 10.5 g. A portion (3.24 g) was coated onto silica (Merck, Art 7734, 38 g) by evaporation of a DMA solution. Chromatography on silica (Merck, Art 15111, 1 kg) with stepwise elution using 5, 10, 15, and 25% EtOH in $CHCl_3$ afforded the product (1.21 g, 32%) as a yellow solid: mp 235–236 °C; NMR (Me_2SO-d_6, 400 MHz) δ 1.29 (t, 3 H, CH_3), 3.26 (t, 1 H, acetylene), 4.24 (q, 2 H, CH_2), 4.33 (d, 2 H, propargyl CH_2), 4.72 (s, 2 H, $CH^9{}_2$), 6.87 (d, 2 H, $H^{3'}$, $H^{5'}$), 7.78 (d, 2 H, $H^{2'}$, $H^{6'}$) plus all quinazoline signals as expected. Anal. ($C_{21}H_{20}N_4O_3$) C, H, N.

4-[*N*-[(2-Amino-4-hydroxy-6-quinazolinyl)methyl]prop-2-ynylamino]benzoic Acid (1c). The ester **4** (0.880 g, 2.34 mmol) was disssolved in Me_2SO (10 mL) and the solution diluted with H_2O (25 mL) and treated with 1 N NaOH (23 mL, 10 mol equiv). The mixture was stirred at 25 °C for 24 h and filtered and the filtrate acidified with 1 N HCl to pH 3.3. The precipitate was centrifuged (14000*g*/30 min) and washed three times by suspension (H_2O, 50 mL)–centrifugation–decantation. The product was dried at 80 °C over P_2O_5 in vacuo, giving a yellow solid: 0.248 g (30%); mp >300 °C dec; NMR, Table I; UV, Table II. Anal. ($C_{19}H_{16}N_4O_3$) C, H, N.

Diethyl *N*-(4-Nitrobenzoyl)-L-aspartate (5d). To a stirred suspension of diethyl L-aspartate hydrochloride (9.03 g, 40 mmol) in toluene (50 mL) cooled to 0 °C was added pyridine (8.1 mL, 100 mmol, 2.5 mol equiv). After the mixture was stirred for 15 min at 0 °C, a solution of 4-nitrobenzoyl chloride (11.13 g, 60 mmol) in toluene (50 mL) was added over 20 min. Stirring was continued for 30 min at 0 °C and then for 1 h at room temperature. The mixture was diluted with further toluene (50 mL) and washed successively with 2 N HCl (2 × 50 mL), 5% $NaHCO_3$ (3 × 50 mL), and H_2O (2 × 50 mL). The organic phase was dried ($MgSO_4$) and the solvent removed in vacuo to give an oil that crystallized on standing. Recrystallization from EtOH–H_2O gave the product: 7.20 g (53%); mp 89–90 °C (lit.[17] mp 87–89 °C); NMR satisfactory. Anal. ($C_{15}H_{18}N_2O_7$) C, H, N.

Ethyl *N*-(4-Nitrobenzoyl)-L-alaninate (5g). In similar manner as for **5d** ethyl L-alaninate hydrochloride (9.22 g, 60 mmol) in toluene (100 mL), pyridine (12.2 mL, 150 mmol), and 4-nitrobenzoyl chloride (16.70 g, 90 mmol) were allowed to react. The mixture was diluted with EtOAc (200 mL) before similar extractive workup. The resulting crude product, a cream-colored solid, was purified by column chromatography on silica gel (Merck, Art 7734), eluting with 30% EtOAc in petroleum ether. A white crystalline solid was obtained: 6.52 g (41%); mp 118–120 °C; NMR satisfactory. Anal. ($C_{12}H_{14}N_2O_5$) C, H, N.

Diethyl *N*-(4-Aminobenzoyl)-L-aspartate (6d). **5d** (6.09 g, 18.0 mmol) in EtOH (150 mL) was hydrogenated at atmospheric pressure with use of a 10% Pd–C catalyst (0.5 g). Filtration and removal of the solvent in vacuo gave an oil that crystallized on standing and was used without further purification: 5.40 g (97%); mp 116–117 °C (lit.[17] mp 116–117 °C; lit.[18] 144–146 °C); NMR

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satisfactory. Anal. ($C_{15}H_{20}N_2O_5$) C, H, N.

Ethyl *N*-(4-Aminobenzoyl)-L-alaninate (6g). In a similar manner to **6d**, **5g** (5.33 g, 20 mmol) gave **6g**: 4.00 g (85%); mp 157–159 °C; NMR satisfactory. Anal. ($C_{12}H_{16}N_2O_3$) C, H, N.

Diethyl *N*-[4-(Prop-2-ynylamino)benzoyl]-L-aspartate (7d). A mixture of **6d** (3.08 g, 10 mmol) 2,6-lutidine (1.39 mL, 12 mmol), and propargyl bromide (1.43 g, 12 mmol) in DMA (15 mL) was stirred at room temperature for 48 h. The mixture was poured into H_2O (100 mL) and extracted with EtOAc (100 mL). The organic phase was washed with H_2O (2 × 100 mL) and dried ($MgSO_4$) and the solvent removed in vacuo. The resulting oil was purified by column chromatography on silica gel (Merck, Art 7734) eluting with 30% EtOAc in CH_2Cl_2 to give **5d** as an oil: 1.85 g (52%); NMR ($CDCl_3$, 90 MHz) δ 2.21 (t, J = 1.5 Hz, 1 H, acetylene), 3.92 (br s, 2 H, propargyl CH_2), 4.35 (br s, 1 H, aniline NH). Anal. ($C_{18}H_{22}N_2O_5 \cdot 0.5H_2O$) C, H, N.

Ethyl *N*-[4-(Prop-2-ynylamino)benzoyl]-L-alaninate (7g). In a similar manner to **7d**, the amine **6g** (2.36 g, 10 mmol) was propargylated but replacing the lutidine with K_2CO_3 (1.38 g, 10 mmol) and eluting with 25% EtOAc in CH_2Cl_2. **7g** was obtained: 1.60 g (58%); mp 84–85 °C; NMR ($CDCl_3$, 90 MHz) δ 2.30 (t, J = 2 Hz, 1 H, acetylene), 4.05 (br s, 2 H, propargyl CH_2), 4.50 (br s, 1 H, aniline NH). Anal. ($C_{15}H_{18}N_2O_3$) C, H, N.

Diethyl *N*-[4-[*N*-[(2-Amino-4-hydroxy-6-quinazolinyl)methyl]prop-2-ynylamino]benzoyl]-L-aspartate (8d). A mixture of **3**[13] (1.42 g, 4.23 mmol), $CaCO_3$ (0.42 g, 4.2 mmol), and **7d** (1.50 g, 4.23 mmol) in DMA (10 mL) was stirred at room temperature for 4 days. The mixture was filtered, and the solids were washed with DMA; evaporation of the combined filtrates in vacuo gave a gum. This was purified by column chromatography on silica gel (Merck, Art 7734), eluting with 10% MeOH in CH_2Cl_2 to give the product: 1.10 g (48%); mp 157–159 °C; NMR (Me_2SO-d_6, 90 MHz) δ 1.18 (t, J = 7 Hz, 6 H, ester CH_3's), 2.82 (m, 2 H, CH_2CO), 3.11 (t, J = 2 Hz, 1 H, acetylene), 4.09 (q, J = 7 Hz, 2 H, ester CH_2), 4.11 (q, J = 7 Hz, 2 H, ester CH_2), 4.28 (br s, 2 H, propargyl CH_2), 4.66 (br s, 2 H, CH^9_2), 4.80 (m, 1 H, CH), 6.86 (d, J = 8 Hz, 2 H, $H^{3'}$, $H^{5'}$), 7.68 (d, J = 8 Hz, 2 H, $H^{2'}$, $H^{6'}$), 8.38 (d, J = 7 Hz, 1 H, amidic NH) plus all quinazoline signals as expected. Anal. ($C_{27}H_{29}N_5O_6 \cdot 1.5H_2O$) C, H, N.

Ethyl *N*-[4-[*N*-[(2-Amino-4-hydroxy-6-quinazolinyl)methyl]prop-2-ynylamino]benzoyl]-L-alaninate (8g). In a similar manner to **8d**, reaction of the propargylamine **7g** (1.50 g, 5.47 mmol) and 1 mol equiv each of **3**[13] and $CaCO_3$ and stirring for 2 days gave, after chromatography, the impure product. Recrystallization from EtOH delivered it pure: 0.45 g (16%); mp 220–225 °C; NMR (Me_2SO-d_6, 90 MHz) δ 1.12 (t, J = 8 Hz, 3 H, ester CH_3), 1.31 (d, J = 8 Hz, 3 H, CH_3), 3.11 (br s, 1 H, acetylene), 4.04 (q, J = 7 Hz, 2 H, ester CH_2), 4.23 (br s, 2 H, propargyl CH_2), 4.31 (m, 1 H, CH), 4.64 (s, 2 H, CH^9_2), 6.77 (d, J = 8 Hz, 2 H, $H^{3'}$, $H^{5'}$), 7.66 (d, J = 8 Hz, 2 H, $H^{2'}$, $H^{6'}$), 8.25 (d, J = 7 Hz, 1 H, amidic NH) plus all quinazoline signals as expected. Anal. ($C_{24}H_{25}N_5O_4 \cdot H_2O \cdot 0.5HBr$) C, H, N, Br.

***N*-[4-[*N*-[(2-Amino-4-hydroxy-6-quinazolinyl)methyl]prop-2-ynylamino]benzoyl]-L-aspartic Acid (1d).** A mixture of **7d** (0.260 g, 0.48 mmol) in 50% EtOH–H_2O (20 mL) was treated with 1 N NaOH (1.5 mL, 1.5 mmol) and the mixture stirred at room temperature for 18 h. Addition of 0.1 N HCl (15 mL, 1.5 mmol) gave a gelatinous precipitate that was purified by five cycles of suspension (H_2O)–centrifugation–decantation. The final aqueous suspension was freeze-dried to give the product as an amorphous white solid: 0.170 g (72%); mp >310 °C; NMR, Table I; UV, Table II. Anal. ($C_{23}H_{21}N_5O_6 \cdot 1.7H_2O$) C, H, N.

***N*-[4-[*N*-[(2-Amino-4-hydroxy-6-quinazolinyl)methyl]prop-2-ynylamino]benzoyl]-L-alanine (1g).** In a similar manner to **1d**, the ester **8g** (0.25 g, 0.49 mmol) and 2.5 mol equiv of base gave the product **1g**: 0.180 g (79%); mp 223–226 °C dec; NMR, Table I; UV, Table II. Anal. ($C_{22}H_{21}N_5O_4 \cdot 2.5H_2O$) C, H. N.

Methyl *N*-(4-Aminobenzoyl)glycinate Hydrochloride (6f). $SOCl_2$ (5.95 g, 50 mmol) was added dropwise with stirring to MeOH (17 mL), keeping the temperature below −10 °C. With this temperature control, 4-aminohippuric acid (9.71 g, 50 mmol) was added and the mixture then allowed to warm to room temperature with stirring. Further MeOH (30 mL) was added and the slurry stirred overnight for 22 h to complete the reaction. The mixture was evaporated to dryness on a rotary evaporator to give the product as a white powder that was dried over P_2O_5 in vacuo: 12.03 g (98%); mp 110–119 °C. Anal. ($C_{10}H_{13}ClN_2O_3$) C, H, N, Cl.

Methyl *N*-[4-(Prop-2-ynylamino)benzoyl]glycinate (7f). A solution of **6f** (4.90 g, 20 mmol) in DMF (200 mL) containing K_2CO_3 (5.52 g, 40 mmol) in suspension was treated with propargyl bromide (80% solution in toluene, 4.76 g, 40 mmol). The mixture was stirred at 110 °C for 3 h and cooled and filtered. The solvent was removed from the filtrate (40 °C (0.4 mm)), and the resulting oil was partitioned between H_2O (1 L) and CH_2Cl_2 (1 L). The organic phase was removed, dried (filtration through phase-separation paper and then using $MgSO_4$), and concentrated in vacuo to give a viscous red oil. This was chromatographed on silica (Merck, Art 15111), eluting with $CHCl_3$ to afford the pure product as a yellow oil that slowly crystallized; 2.49 g (50.5%). The analytical sample was recrystallized from H_2O–MeOH (2:1) as pale yellow needles: mp 119–120 °C; NMR ($CDCl_3$, 60 MHz) δ 2.25 (t, 1 H, acetylene), 3.93 (d, 2 H, propargyl CH_2). Anal. ($C_{13}H_{14}N_2O_3$) C, H, N.

Methyl *N*-[4-[*N*-[(2-Amino-4-hydroxy-6-quinazolinyl)methyl]prop-2-ynylamino]benzoyl]glycinate (8f). A mixture of **7f** (0.135 g, 0.55 mmol), **3**[13] (0.184 g, 0.55 mmol), and $CaCO_3$ (0.22 g, 2.2 mmol) in DMA (5 mL) was stirred for 72 h at room temperature. The mixture was filtered, and the filtrate and washings were evaporated to dryness at 40 °C (0.1 mm) to give a gum. This was chromatographed on silica (Merck, Art 15111), eluting with 8% MeOH in CH_2Cl_2 to afford the product as an off-white solid that was dried over P_2O_5 in vacuo: 0.087 g (38%); mp 234–236 °C; NMR (Me_2SO-d_6, 250 MHz) δ 3.19 (t, 1 H, acetylene), 3.64 (s, 3 H, ester CH_3), 3.96 (d, 2 H, glycine CH_2), 4.28 (d, 2 H, propargyl CH_2), 4.67 (s, 2 H, CH^9_2), 6.85 (d, 2 H, $H^{3'}$, $H^{5'}$), 7.72 (d, 2 H, $H^{2'}$, $H^{6'}$), 8.60 (t, 1 H, amidic NH), plus all quinazoline signals as expected. Anal. ($C_{22}H_{21}N_5O_4$) C, H, N.

***N*-[4-[*N*-[(2-Amino-4-hydroxy-6-quinazolinyl)methyl]prop-2-ynylamino]benzoyl]glycine (1f).** The ester **8f** (0.190 g, 0.45 mmol) suspended in 30% EtOH–H_2O (10 mL) was treated with 1 N NaOH (1.80 mL, 4 mol equiv) and the resulting solution then kept at 25 °C for 4 h. The solution was clarified by filtration and acidified to pH 3.4 with 1 N HCl to give a gelatinous precipitate. Centrifugation at 20000*g* followed by two wash cycles of resuspension (H_2O)–centrifugation–decantation and then drying over P_2O_5 at room temperature gave the product as an amorphous solid: 0.150 g (77%); mp 246–250 °C; NMR, Table I; UV, Table II. Anal. After drying at 100 °C ($C_{21}H_{19}N_5O_4 \cdot 1.2H_2O$) C, H, N.

Methyl 4-[4-(Prop-2-ynylamino)phenyl]butyrate (10). A solution of the amine **8**[15] (4.83 g, 25 mmol) in MeOH (100 mL) containing K_2CO_3 (3.46 g, 25 mmol) in suspension was stirred at 25 °C, and to it, during 10 min, was added a solution of propargyl chloride (9.31 g, 125 mmol) in MeOH (50 mL). The mixture was refluxed for 3 h and cooled and filtered. The filtrate was concentrated to dryness and the resulting solid partitioned between CH_2Cl_2 (1 L) and H_2O (1 L). The organic phase was removed, dried ($MgSO_4$), and concentrated to dryness to give a yellow oil. This was chromatographed on silica (Merck, Art 15111), eluting with 10% EtOAc–petroleum ether to give the pure product (1.50 g, 26%) as a viscous yellow oil: NMR (CCl_4, 60 MHz) δ 2.07 (t, 1 H, acetylene), 3.7 (br s, 1 H, aniline NH), 3.80 (d, J = 2.3 Hz, 2 H, propargyl CH_2). Anal. ($C_{14}H_{17}NO_2$) C, H, N.

Methyl 4-[4-[*N*-[(2-Amino-4-hydroxy-6-quinazolinyl)methyl]prop-2-ynylamino]phenyl]butyrate (11). A mixture of **10** (0.293 g, 1.3 mmol), **3**[13] (0.436 g, 1.3 mmol), and $CaCO_3$ (0.52 g, 5.2 mmol) in DMA (10 mL) was stirred for 25.5 h at 25 °C. The mixture was filtered with washings and the combined filtrate concentrated at 60 °C (0.5 mm). The resulting yellow solid was chromatographed on silica (Merck, Art 15111), eluting with 6% MeOH in CH_2Cl_2 to afford the product as an off-white solid: 0.157 g (29.8%); mp 205–209 °C; NMR (Me_2SO-d_6, 250 MHz) δ 1.76 (quintet, 2 H, butyr CH^3_2), 2.28 (t, 2 H, CH_2), 2.46 (t, 2 H, CH_2), 3.13 (t, 1 H, acetylene), 3.57 (s, 3 H, ester CH_3), 4.09 (d, 2 H, propargyl CH_2), 4.49 (s, 2 H, CH^9_2), 6.77 (d, 2 H, $H^{3'}$, $H^{5'}$), 7.00 (d, 2 H, $H^{2'}$, $H^{6'}$) plus all quinazoline signals as expected. Anal. ($C_{23}H_{24}N_4O_3$) C, H, N.

4-[4-[*N*-[(2-Amino-4-hydroxy-6-quinazolinyl)methyl]prop-2-ynylamino]phenyl]butyric Acid (1e). 11 (0.303 g, 0.75 mmol) was stirred in a mixture of H_2O (12.5 mL), EtOH (2.5 mL), and 1 N NaOH (3.00 mL, 4 mol equiv) for 20 min whereupon a

solution was obtained. It was kept at 25 °C for 18 h, clarified by filtration, and acidified with 0.1 N HCl. The resulting white precipitate was freed from inorganic ions by three cycles of suspension (H_2O, 100 mL)–centrifugation–decantation. The product was dried over P_2O_5 in vacuo at 25 °C: 0.26 g (89%); mp 271 °C dec; NMR, Table I; UV, Table II. Anal. After drying at 150 °C ($C_{22}H_{22}N_4O_3$) C, H, N.

Biological Evaluation

The analogues **1c–g** and also diethyl N^{10}-propargyl-5,8-dideazafolate (**1b**)[8] were tested for their inhibition of purified L1210 thymidylate synthase as previously described.[19] For determination of the inhibition of the growth of L1210 cells in culture the compound was suspended in unsupplemented RPMI 1640 medium that was then adjusted to about pH 9.0 with 1 N NaOH to aid dissolution. After sterile Millipore filtration (0.22 μm) the pH was adjusted back to about 7.5 with sterile 1 N HCl and 0.5 mL of the resulting solution added to 4.5 mL of a suspension of L1210 cells (1×10^5 mL^{-1}) and cultured as previously described.[8] The results are expressed in Table III.

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Registry No. **1b**, 76858-74-7; **1c**, 101248-32-2; **1d**, 80014-97-7; **1e**, 101248-33-3; **1f**, 101248-34-4; **1g**, 101248-35-5; **2**, 101248-36-6; **3**, 77766-62-2; **4**, 101248-37-7; **5d**, 27128-94-5; **5g**, 76765-75-8; **6d**, 5854-13-7; **6f**·HCl, 101248-38-8; **6g**, 76765-77-0; **7d**, 80014-88-6; **7f**, 101248-39-9; **7g**, 101248-40-2; **8d**, 80014-78-4; **8f**, 101248-41-3; **8g**, 101248-42-4; **9**, 20637-09-6; **10**, 101248-43-5; **11**, 101248-44-6; HC≡CCH$_2$Cl, 624-65-7; HC≡CCH$_2$Br, 106-96-7; diethyl L-aspartate hydrochloride, 16115-68-7; 4-nitrobenzoyl chloride, 122-04-3; ethyl L-alaninate hydrochloride, 1115-59-9; 4-aminohippuric acid, 61-78-9; thymidylate synthase, 9031-61-2.

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Notes

Indolo[2,1-*c*][1,4]benzodiazepines: A New Class of Antiallergic Agents

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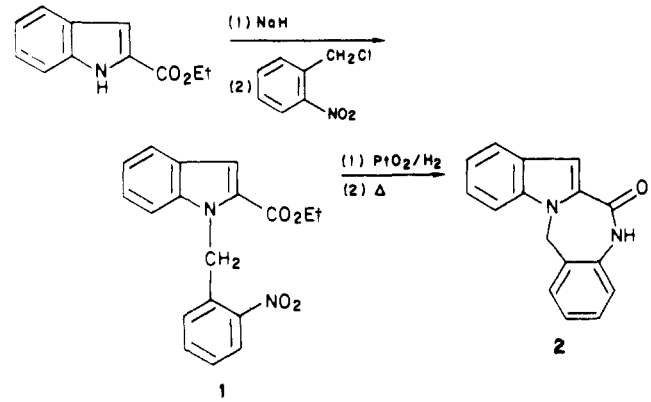
A series of 12-(cyclic alkylamino)-6*H*-indolo[2,1-*c*][1,4]benzodiazepines were synthesized that possess antihistamine and antiserotonin activities as well as ability to inhibit mediator release. Compound **6a**, 12-(4-methyl-1-piperazinyl)-6*H*-indolo[2,1-*c*][1,4]benzodiazepine was a more potent inhibitor of serotonin release than disodium cromoglycate (DSCG) and ketotifen andd approximately equivalent to oxatomide. In the in vivo tests (PCA and ALA), compound **6a** was equivalent or superior to DSCG and oxatomide. These agents have potential for the treatment of a variety of allergic conditions.

The clinical success of disodium cromoglycate (DSCG)[1] in the prophylactic treatment of asthma has stimulated a substantial research effort to discover more potent and orally active inhibitors of mediator release from mast cells and basophils. Consequently, numerous compounds have been described to possess desired preclinical biological properties, and many of these have been proposed to be orally antiallergic agents.[2] The mechanisms of these agents are varied and, in many cases, not totally elucidated. Some have been reported to be pure mediator-release inhibitors (e.g., bufrolin[3]), while others also possess the ability to block receptors for the mediators histamine, serotonin, and/or leukotrienes (e.g., oxatomide,[4] ketotifen,[5] FPL-55712[6]).

Scheme I



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