N-(2-[2-(2,4-Diamino-6-quinazolinyl)ethyl]benzoyl)-L-glutamic Acid

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A trideaza analog of aminopterin, *N*-(4[2-(2,4-diamino-6-quinazolinyl)ethyl]benzoyl)-L-glutamic acid, was prepared by a Wittig condensation of 2,4-diaminoquinazoline-6-carboxaldehyde and [*P*(*N*-[1,3-bis(ethoxycarbonyl)propan-1-yl]aminocarbonyl)phenylmethyl]triphenylphosphonium bromide followed by catalytic reduction and mild hydrolysis. This compound was found to have confirmed inhibitory activity against leukemia L1210 in mice.

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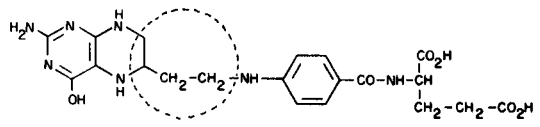
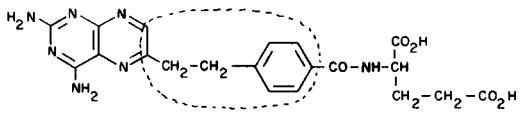
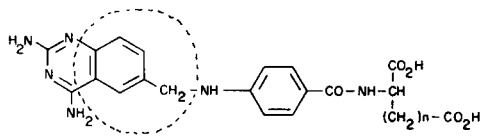
In addition to general considerations of inhibition of tetrahydrofolate-dependent nucleic acid and amino acid syntheses in the design of antifolates (1a-3), a comparison of the structures of some existing antifolic agents such as tetrahydrohomofolic acid (I) (4-7), 10-deazaaminopterin (II) (8), and quinazoline derivatives (IIIa and IIIb) (9-13) reveals that lipophilicity of the center portion linking the aminopyrimidinyl terminal and the amino acid terminal may be a contributing factor to antifolate activity. In other words, whereas both polar ends of the molecule are understandably involved in binding with enzymes and other biological macromolecules, the center portion is rather lipophilic and it appears that the lipophilicity of the center linkage may be in direct proportion to the antifolate activity. This postulation is also supported by



I



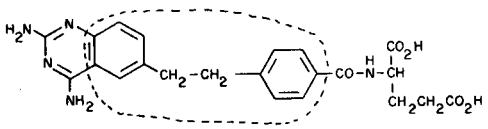
II



IIIa, n=1

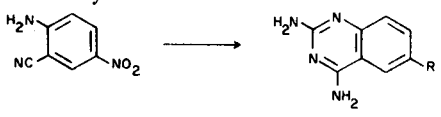
b, n=2

reports describing greater antileukemic activity of 3′,5′-dichloromethotrexate than that of methotrexate and greater activity of 5-chloro- or 5-methylquinazoline antifoles than that of the 5-unsubstituted compounds. Consequently, synthesis of 5,8,10-trideazaaminopterin (IV) was conducted.



IV

One of the key intermediates, 2,4-diaminoquinazoline-6-carboxaldehyde (VIe), was prepared according to the procedure of Davoll and Johnson (14) with some modifications. Condensation of 2-amino-5-nitrobenzonitrile (V) with guanidine gave the 6-nitroquinazoline VIa in 73% yield. Reduction of VIa was originally carried out in dimethylformamide in the presence of palladium-on-charcoal which required several hours for completion (14). In addition, dimethylformamide had to be quickly evaporated after the reduction since prolonged standing of the hydrogenated reaction mixture often caused a decrease in yield of the desired compound VIb. In our hands it was found that addition of a small amount of acetic acid to the mixture prior to hydrogenation drastically cut down the time required for completion. Also, the product VIb can be readily isolated from the hydrogenated mixture by simply pouring the latter into a large volume of ethyl acetate followed by filtration. The yield by this modified method was increased from 64% to 94%. Diazotization of the triaminoquinazoline VIb followed by treatment with cuprous cyanide yielded the 6-cyano derivative VIc, which was converted to the 6-carboxaldehyde VIe by Raney nickel catalytic hydrogenation via the hydrazone VId.



V

VIa, R = NO_2

b, R = NH_2

c, R = CN

d, R = CH=N-NH-C_6H_5

e, R = CHO

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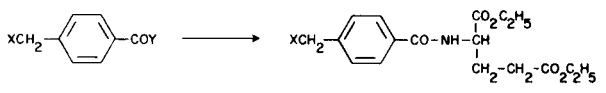
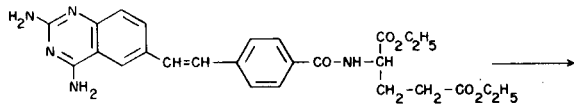
The other intermediate, VIIIb, for the proposed Wittig condensation was prepared as follows: Bromination of *p*-toluoyl chloride VIIa with bromine at 170-180° under light (15,16) gave a 77% yield of the dibromo derivative VIIb. Treatment of VIIb with diethyl L-glutamate hydrochloride and two equivalents of triethylamine in methylene chloride at 0° yielded the diester VIIIa. The latter was converted to its phosphonium bromide VIIIb with triphenylphosphine in refluxing toluene.



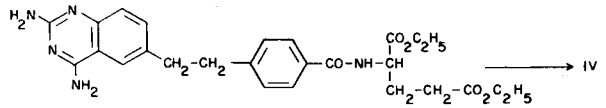
VIIa, X = H, Y = Cl
b, X, Y = Br

VIIIa, X = Br
b, X = $(C_6H_5)_3P^+Br^-$

The Wittig condensation of compounds VIe and VIIIb was carried out by stirring the phosphonium bromide VIIIb in sodium hydride in dimethylformamide followed by addition of VIe. The reaction was completed in 4 days at room temperature to form IX, which was hydrogenated catalytically to give compound X. Hydrolysis of X with four equivalents of potassium carbonate in aqueous ethanol at room temperature for 3 days afforded the desired product IV.



IX



X

Preliminary test results of compound IV in the screening program of the National Cancer Institute indicated that the compound possessed confirmed inhibitory activity against leukemia L1210 in mice with T/C values above 130 at dosage as low as 0.08 mg./kg. At 6.25 mg./kg., the T/C value was 170.

EXPERIMENTAL

Melting points were taken on a Thomas-Hoover melting point apparatus. The infrared spectra were taken with a Perkin-Elmer Infracord and the NMR spectra data were determined on a Varian EM 360A spectrophotometer.

2,4-Diamino-6-nitroquinazoline (VIa).

To a solution of 216 ml. of 25% sodium methoxide in methanol (Aldrich) and 800 ml. of absolute ethanol at 0-5° was added 96 g. (1 mole) of guanidine hydrochloride. The cloudy mixture was stirred at the same temperature for 30 minutes and filtered through Celite. The clear solution was transferred into a 2-liter round bottom flask to which was added 163 g. (1 mole) of 2-amino-5-nitrobenzonitrile. The mixture was refluxed and stirred for 5 hours, then allowed to stand at room temperature overnight. The resulting orange brown solid was collected by filtration, and recrystallized from acetic acid to yield the acetate of VIa as yellow needles, which was washed throughly with ethanol and dried to give 150 g. (73% yield) of product, m.p. > 330° [lit. (14) m.p. > 360°].

2,4,6-Triaminoquinazoline (VIb).

A mixture of 25 g. of VIa, 200 ml. of dimethylformamide, 2 ml. of acetic acid and 2.5 g. of 10% palladium-on-charcoal was hydrogenated under 4.2 kg./cm.^{2} of hydrogen. The hydrogenation was completed within 90 minutes. The catalyst was removed by filtration and the filtrate poured, with stirring, into 1,400 ml. of ethyl acetate. After standing overnight at 5°, the product was collected by filtration to give 20.2 g. (94% yield) of VIb as a yellow solid.

2,4-Diaminoquinazoline-6-carbonitrile (VIc).

To a solution of 63 g. (0.36 mole) of VIb in 750 ml. of 2 *N* hydrochloric acid cooled at 2° in an ice-water bath was added during a 2 minutes period a cold solution of 26 g. of sodium nitrite in 180 ml. of water. The mixture was stirred at 2° for 20 minutes then added to a mechanically stirred solution of cuprous cyanide (prepared in a 4-liter beaker by the addition of 84.6 g. of cupric sulfate pentahydrate in 270 ml. of water to a solution of 101 g. of potassium cyanide in 180 ml. of water) at 39° in a period of 10 minutes. The mixture was stirred vigorously at 50-55° for 30 minutes, then cooled to 40°, followed by treatment of the resulting mixture with 360 ml. of concentrated ammonium hydroxide. After standing at room temperature for 1 hour, the precipitate was collected by filtration, washed with water, and extracted with 1 liter of 15% boiling aqueous acetic acid. The solution was filtered while hot. The filtrate was diluted with 800 ml. of 2-ethoxyethanol and basified with 300 ml. of concentrated ammonium hydroxide. The resulting precipitate was collected by filtration and washed with water to give 26.5 g. (40% yield) of VIc, m.p. > 300° [lit. (14) m.p. > 360°].

2,4-Diaminoquinazoline-6-carboxaldehyde Phenylhydrazone (VId).

A mixture of 7.4 g. (0.04 mole) of the cyanide VIc, 250 ml. of 50% aqueous acetic acid, 4.8 ml. of phenylhydrazine, and 15 g. of activated Raney nickel was hydrogenated under 4.2 kg./cm.^{2} of hydrogen. After 0.05 mole of hydrogen was consumed (*ca.* 2 hours), the hydrogenation was discontinued and the reaction mixture was heated to boiling and finally filtered through Celite while hot. On cooling, the yellow green solid was collected by filtration, washed with dilute acetic acid and dried to give 9 g. (51% yield) of crude VId, m.p. 223-236° [lit. (14) m.p. 232-234°]. This crude product was used for the preparation of the aldehyde VIe without further purification.

2,4-Diaminoquinazoline-6-carboxaldehyde (VIe).

A mixture of 16 g. (0.047 mole) of VId, 8.5 g. of *p*-nitrobenzaldehyde and 400 ml. of 50% aqueous acetic acid was refluxed for 2 hours. The reaction mixture was cooled and the dark red solid was removed by filtration. The red filtrate was stirred with 2 g. of activated charcoal overnight, filtered, and evaporated. The residue was suspended in 100 ml. of water and basified with 10% sodium carbonate. The resulting light red solid was collected by filtration and washed with water. It was then boiled with ethanol

(2 x 50 ml.) to remove the red impurity to give 7.7 g. (87% yield) of VIe, m.p. >300° [lit. (14) m.p. >360°].

Anal. Calcd. for $C_9H_8N_4O$: C, 57.44; H, 4.28; N, 29.77. Found: C, 57.16; H, 4.40; N, 29.69.

p-(Bromomethyl)benzoyl Bromide (VIIb).

To 85 g. (0.557 mole) of *p*-toluoyl chloride (VIIa) at 160° was added, with stirring, 28.8 ml. (0.557 mole) of bromine. Throughout the addition, which took 90 minutes, the reaction was illuminated with a tungsten lamp. Illumination was continued for 30 minutes after the addition. The resulting red solution was cooled to 70° and was distilled under reduced pressure. The fraction boiling between 141-144°/2.5 mm was collected. The yield of VIIb was 119.3 g. (77%) [lit. (15) b.p. 170-171°/20 mm].

Diethyl *N*-[*p*-Bromomethyl)benzoyl]-L-glutamate (VIIIa).

To a solution of 11 g. (0.039 mole) of VIIb in 25 ml. of methylene chloride cooled in an ice water bath was added dropwise a mixture of 9.5 g. (0.039 mole) of diethyl L-glutamate hydrochloride and 8 g. (0.08 mole) of triethylamine in 30 ml. of methylene chloride in a period of 30 minutes. The resulting mixture was stirred in the ice water bath for an additional 3 hours, then at room temperature for 2 hours. It was diluted with 25 ml. of methylene chloride and washed successively with 0.1 *N* hydrochloric acid (75 ml.), 1% sodium carbonate (100 ml.) and twice with saturated sodium chloride solution. After being dried overnight over magnesium sulfate, the solution was evaporated to give 12.5 g. (78% yield) of a white solid, m.p. 90-95°. Recrystallization from aqueous ethanol (1:1) yielded an analytical sample, m.p. 102-105°. $[\alpha]_D^{24°}$ = +18.7° (C = 2.3, chloroform); λ max 3320 cm^{-1} (NH), 1725 and 1630 cm^{-1} (2CO); nmr (deuteriochloroform): δ 0.1-1.9 (2*t*, 6H, $2CH_3$), 1.9-2.8 (*m*, 4H, $-(CH_2)_2-$), 3.75-4.95 (*m*, 7H, $2CO_2CH_2CH_3$, CH, and $C_6H_4CH_2-$), and 7.1-7.9 (*m*, 4H, ring Hs).

Anal. Calcd. for $C_{17}H_{22}BrNO_5$: C, 51.01; H, 5.54; N, 3.50. Found: C, 51.39; H, 5.72; N, 3.75.

[*P*(*N*-[1,3-Bis(ethoxycarbonyl)propan-1-yl]aminocarbonyl)phenylmethyl]thiphenylphosphonium Bromide (VIIIb).

A mixture of 20 g. (0.05 mole) of VIIIa and 13.3 g. (0.05 mole) of triphenylphosphine in 150 ml. of toluene, was refluxed with stirring for 1 hour. The solids dissolved almost immediately followed by the formation of a precipitate. The mixture was cooled and the white solid was collected by filtration, washed with ether, then dried *in vacuo* to give 17 g. (50% yield) of VIIIb, m.p. 208-213° dec.; λ max: 3170, 1725 and 1645 cm^{-1}; nmr (deuteriochloroform): δ 1.25 (6H, J = cps, $2CH_3$), 2.20-2.65 (*m*, 4H, CH_2CH_2), 4.10-4.35 (*q*, 4H, J = 7 cps, 2 ethoxy CH_2), 4.35-4.95 (*m*, 1H, CH), 5.45 (*d*, 2H, J = 16 cps, $C_6H_4CH_2$), 6.85-8.35 (*m*, 4H, aromatic Hs).

Diethyl *N*-(4-[2,4-Diamino-6-quinazolinyl)ethylenyl]benzoyl)-L-glutamate (IX).

Both reactants VIe and VIIIb were dried in a vacuum oven at 60° for 4 hours and stored overnight in a desiccator (calcium chloride) before use. The solvent, dimethylformamide, was dried over phosphorus pentoxide and distilled.

To a suspension of 0.91 g. (16 mmoles) of sodium hydride (50% in mineral oil) in 20 ml. of dimethylformamide was added a solution of 12.6 g. (19 mmoles) of VIIIb in 110 ml. of dimethylformamide. The mixture was stirred at room temperature for 2 hours. To the resulting yellow suspension was added 3.0 g. (16 mmoles) of VIe and the mixture was stirred at room temperature under nitrogen for 5 days. The light brown reaction mixture was poured, with stirring, into 700 ml. of saturated sodium chloride solution whereupon a yellow brown paste was formed. The paste was separated from the supernatant liquid, washed with water, and dissolved in 100 ml. of chloroform. After drying over anhydrous magnesium sulfate, the chloroform solution was concentrated to 30 ml. and column chromatographed over 100 g. of silica gel (Woelm, Act I, 0.063-0.1 mm) and eluted successively with 400 ml. of chloroform, 650 ml. of 5% methanol in chloroform, and 1,250 ml. of 10% methanol in chloroform to give 5.8 g. (73% yield) fo crude IX, m.p. 187-189°. An analytical sample was obtained by three recrystallizations from ethanol, m.p. 189-190°, $[\alpha]_D^{24°}$ = -47° (C = 1.25, chloroform). λ max: 3650-2700, 1720 and 1620 cm^{-1}; nmr (DMSO-d_6-$CDCl_3$): δ 1.00-1.40 (2*t*, 6H, $2CH_3$), 2.20-2.70 (*m*, 4H, CH_2CH_2), 3.80-4.70 (*m*, 5H, CH, $2CO_2CH_2CH_3$), 6.30 (broad, 1H, NHCO), 7.00-8.85 (*m*, 9H, aromatic and olefinic Hs).

Anal. Calcd. for $C_{26}H_{29}N_5O_5 \cdot 0.5H_2O$: C, 62.39; H, 6.04; N, 13.99. Found: C, 62.21; H, 6.16; N, 14.01.

Diethyl *N*-(4-[2-(2,4-Diamino-6-quinazolinyl)ethyl]benzoyl)-L-glutamate (X).

A mixture of 7.2 g. (14.4 mmoles) of IX in 200 ml. of acetic acid was hydrogenated at 4.2 kg./$cm.^2$ with 1.8 g. of 10% palladium-on-charcoal until the theoretical amount of hydrogen was absorbed (*ca.* 30 hours). The catalyst was removed by filtration and the filtrate was evaporated *in vacuo* to dryness. To the residue was added 200 ml. of ethanol. The mixture was evaporated to remove traces of acetic acid. This operation was repeated two more times. The residue was then dissolved in 40 ml. of ethanol and diluted with 60 ml. of water. The *p*H of the resulting solution was adjusted to 7 with 2% sodium carbonate, whereupon a gray solid was formed. It was isolated and dissolved in 40 ml. of ethanol and column chromatographed on 100 g. of silica gel (Woelm, 70-23 mesh). The column was eluted with 250 ml. of chloroform to yield a black solution, which was discarded. This was followed by eluting with 220 ml. of 5% methanol in chloroform and 1,750 ml. of 10% methanol in chloroform to give 5.0 g. (70% yield) of a compound X. Recrystallization from ethanol-ethyl acetate (1:4) gave analytically pure X, m.p. 190°. λ max: 3400, 3050, 1720, 1625 and 1550 cm^{-1}; nmr (trifluoroacetic acid): δ 1.28 (2*t*, 6H, $2CH_3$), 2.65 (*m*, 4H, $-CH_2CH_2CO$), 3.20 (*s*, 4H, $2CO_2CH_2CH_3$), 5.00 (*m*, 1H, CH), 7.10-8.30 (*m*, 7H, aromatic Hs), 9.30 (broad, 1H, NHCO).

Anal. Calcd. for $C_{26}H_{31}N_5O_5$: C, 63.27; H, 6.33; N, 14.19. Found: C, 63.17; H, 6.44; N, 14.29.

N-(4-[2-(2,4-Diamino-6-quinazolinyl)ethyl]benzoyl)-L-glutamic Acid (IV).

Compound X (2.4 g., 4.9 mmoles) was dissolved in 220 ml. of ethanol and diluted with 120 ml. of water. To this solution was added 9.6 ml. of 1 *M* potassium carbonate at room temperature. The mixture was stirred at room temperature for 3 days. It was concentrated under reduced pressure at <20° to one-half of its original volume. The resulting solution was cooled in an ice bath and carefully neutralized with 0.5 *N* hydrochloric acid. The mixture was allowed to stay at 5° overnight. The precipitate was collected by filtration, washed with water, and dried *in vacuo* to give 1.4 g. (78% yield) of IV as a white solid, m.p. 260° dec.; λ max: 1710, 1680 and 1630 cm^{-1}; nmr (trifluoroacetic acid): δ 2.75 (*m*, 4H, CH_2CH_2CO), 3.20 (*s*, 4H, CH_2CH_2), 5.10 (*m*, 1H, CH), 7.20-8.30 (*m*, 7H, aromatic Hs), and 9.30 (broad, 1H, NHCO).

Anal. Calcd. for $C_{22}H_{23}N_5O_5 \cdot 0.5\ H_2O$: C, 59.18; H, 5.42; N, 15.69. Found: C, 59.31; H, 5.56; N, 15.60.

Acknowledgment.

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