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Synthesis and Biological Activity of 10-Thia-10-deaza Analogs of Folic Acid, Pteroic Acid, and Related Compounds†

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The 10-thia analogs of pteroic acid, folic acid, their esters, and their 4-amino analogs were synthesized through a reaction sequence involving, as a key step, the condensation of 2-amino-3-cyano-5-chloromethylpyrazine with appropriately substituted thiols. The abilities of the products to inhibit the growth of methotrexate (MTX)-sensitive and MTX-resistant microorganisms were investigated as were their abilities to inhibit dihydrofolic acid reductase and thymidylic acid synthetase. Several compounds had high activity.

Since the first reports that folic acid antagonists are useful in the treatment of human neoplastic disease,[1,2] a great deal of research has been centered on investigating the functions and interactions of folic acid and its reduced derivatives in the hope that clues for the synthesis of more effective antagonists might be obtained. Further interest has been added to this research area by the usefulness of folic acid antagonists in the treatment of psoriasis[3,4] and their usefulness in suppressing the immune response[5,6] and in treating protozoal disease.[7]

It is well understood that the functions of tetrahydrofolic acid in essential one-carbon transfer reactions require the availability of amine nitrogens in the 5 and 10 positions of the reduced coenzyme.[8] However, in spite of the essential nature of these nitrogens relatively few attempts in preparing antagonists of folic acid or its reduction products have been centered on replacing them with other atoms.

In homofolic acid and related compounds the distance between these nitrogens was lengthened.[9] In the 10-oxa analog of pteroic and folic acid the 10-nitrogen was replaced by an oxygen.[10] In isofolic acid, the positions of the methylene and amine groups separating the pteridine and benzene rings have been reversed.[11] In 10-deazaaminopterin, the 10-nitrogen of the 4-amino derivative of folic acid was replaced by a methylene group.[12,13] Several of these antagonists showed interesting inhibitory activity in systems requiring folic acid or its reduction products.

The present study describes the synthesis of analogs of folic acid and of pteroic acid, as well as that of their 4-amino analogs, in which the nitrogen in the 10 position was replaced with sulfur. It was hoped that these compounds might have useful inhibitory activity in folic acid requiring systems. The synthesis is outlined as shown in Schemes I and II.

4,4′-Dithiobis(benzoic acid) (1) was synthesized by the procedure of Campaigne and Meyer[14] and converted to the diethyl ester **2**. An attempt to synthesize the diethyl ester directly from ethyl *p*-aminobenzoate yielded material contaminated with a highly explosive by-product, possibly a diazonium intermediate. The explosion occurred while the product was being dried. Reduction of **2** with sodium borohydride yielded the thiol **3**; the reaction was monitored by the uv spectrum change[14] from λ_{max} 273 nm to λ_{max} 332 nm. Condensation of **3** with 2-amino-3-cyano-5-chloromethylpyrazine[15] gave rise to the formation of **4** in excellent yield. The 2-amino-3-cyanopyrazine **4** could then be cyclized with guanidine to form ethyl 4-amino-4-deoxy-10-thia-10-deazapteroate (**6**). The methyl ester **5** could be ob-

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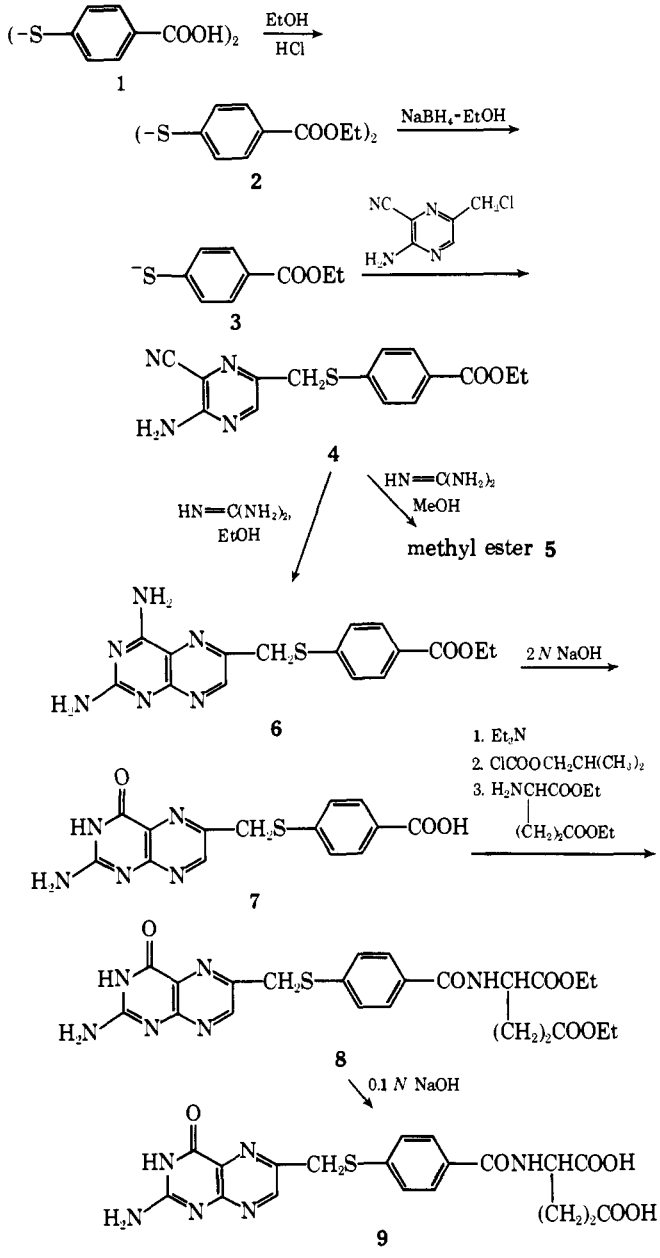
Scheme I



tained when this reaction was carried out in methanol in the presence of an excess of sodium methoxide. Hydrolysis of the ethyl ester yielded the 10-thia analog of pteroic acid (**7**). Condensation of this product with diethyl L-glutamate via the mixed anhydride method using isobutyl chloroformate[9] gave rise to the formation of diethyl 10-thiafolate (**8**). Hydrolysis then yielded the 10-thia analog of folic acid (**9**). In Figure 1, the NMR spectra of **9** and of folic acid are compared.

In a different approach to this synthesis (Scheme II), the condensation of diethyl L-glutamate with 4,4′-dithio(bisbenzoic acid), in the presence of DCC in acetonitrile, formed 4,4′-dithiobis(*N*-benzoyl-L-glutamate) (**10**), from which the corresponding thiol was obtained by reduction with sodium borohydride. The reduction product was permitted to condense with 2-amino-3-cyano-5-chloromethylpyrazine to yield **11**. Cyclization with guanidine now formed diethyl 10-thiaaminopterin, from which the 10-thia analog of aminopterin could be obtained by hydrolysis in weak base.

It should be noted that the reaction sequence followed in Scheme II, but not the reaction sequence followed in Scheme I, led to racemization of the glutamic acid residue following guanidine cyclization.[16]

Discussion

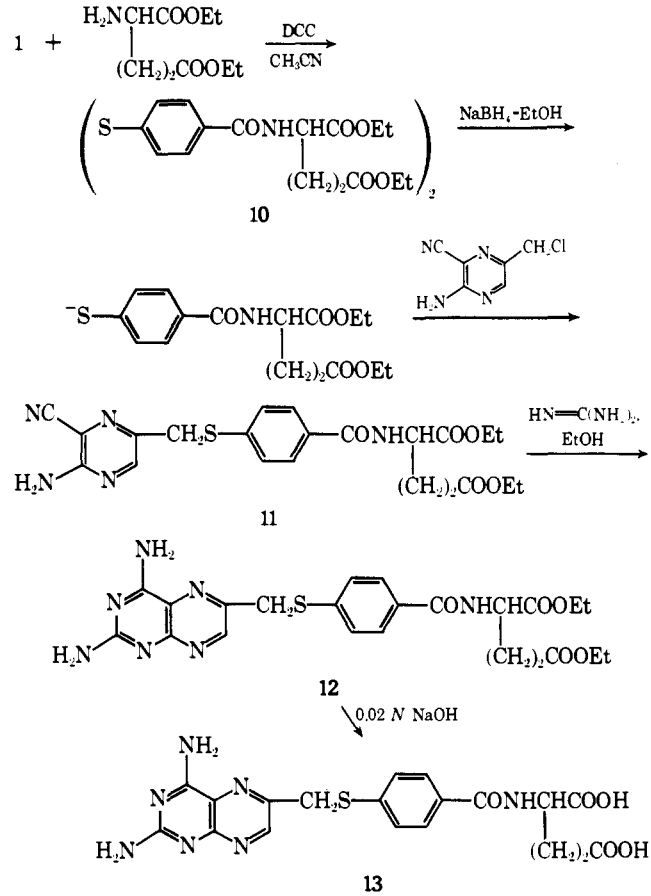
The potencies of the compounds synthesized were tested in the *Streptococcus faecium* and *Lactobacillus casei* systems. As can be seen in Table I, several of these compounds have considerable activity in inhibiting the growth of these organisms. It is of some interest to note that, in addition to several highly active 2,4-diaminopteridine derivatives, the 10-thia analog of folic acid, a 2-amino-4-oxo derivative, exhibited some antibacterial activity as well. These findings are in agreement with reports that 10-deazapteroic acid[17] and 10-deazafolic acid[12] are inhibitors of *S. faecium*. The relatively high in vitro inhibitor activity of the esters of some of the derivatives is not surprising in view of the report many years ago,[18] recently confirmed,[19,20] that esters of amethopterin can have considerable activities.

The ability of some of the esters (**5, 6, 12**) to inhibit the growth of a methotrexate (MTX)-resistant strain of *S. faecium* is of some potential interest. It may be speculated that this is due to these esters, but not the corresponding acids, being able to enter resistant cells.

Several of the compounds were also tested for their abilities to induce 50% inhibition of dihydrofolic acid reductase and thymidylic acid synthetase. These data are summarized in Table II. None of the compounds were effective inhibitors of thymidylate synthetase; however, the ability of the racemic 10-thia analog of aminopterin to inhibit dihydrofolic acid reductase approaches that of MTX.

Studies of the abilities of several of these derivatives to inhibit the growth of leukemia cells in tissue culture are in progress in the laboratory of Dr. Ming-Yü Chu at Brown University.

Scheme II



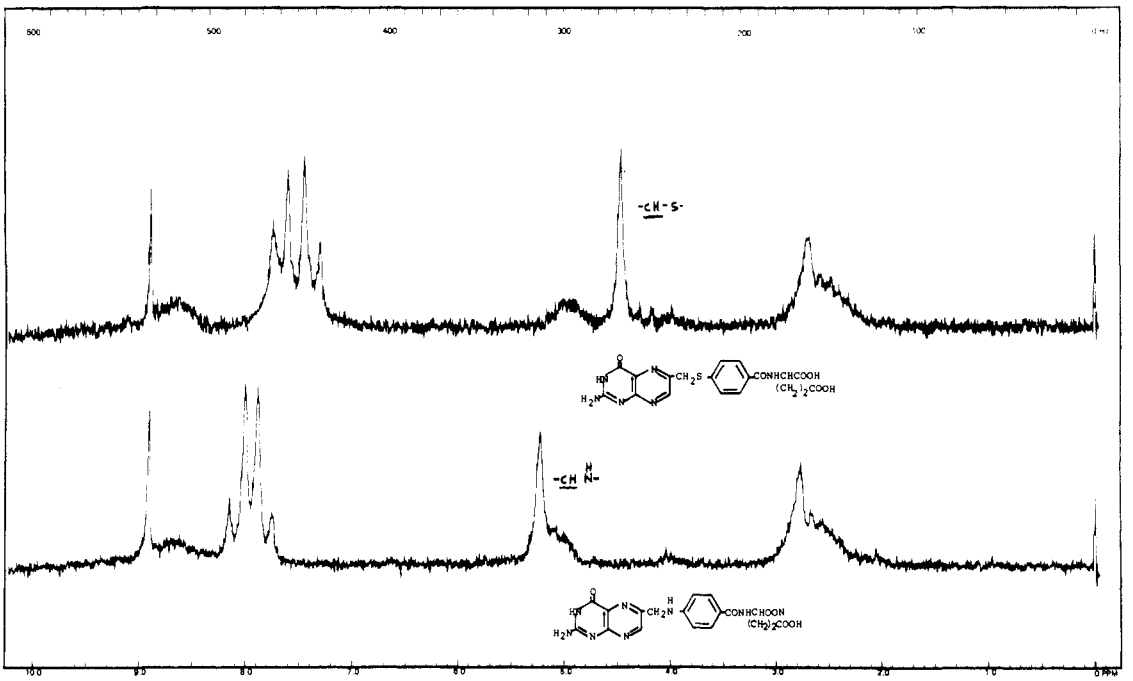


Figure 1. NMR spectrum (F_3CCOOH) of 10-thiafolic acid compared with that of folic acid (Calbiochem).

Experimental Section

Melting points were determined with a Gallenkamp melting point apparatus and are not corrected. Uv spectra were determined with a Cary Model 14 spectrophotometer, NMR spectra were measured in $CDCl_3$, in DMSO-d_6, and in TFA, using TMS as an internal reference standard with a Hitachi Perkin-Elmer Model R-20B spectrometer. Elemental analyses were carried out by Dr. H. Agahigian, Baron Consulting Co., Orange, Conn., and obtained within 0.4% of the theoretical values.

Diethyl 4,4′-Dithiobisbenzoate (2). 4,4′-Dithiobis(benzoic acid)(1) was prepared by the method of Campaigne and Meyer.[14] The acid was esterified with ethanol–HCl. The product was recrystallized from ether (at −70°): mp 53–56°; uv λ_{max} (EtOH) 273 nm (ϵ 32,500); NMR ($CDCl_3$) δ 1.34 (t, 3 H, $-CH_2CH_3$), 4.23 (q, 2 H, $-CH_2CH_3$), 7.42 and 7.88 (aromatic quartet, 4 H, J = 8.4 Hz). Anal. ($C_{18}H_{18}O_2S$) C, H.

2-Amino-3-cyano-5-[(*S*-*p*-carbethoxyphenyl)thiomethyl]-pyrazine (4). Diethyl 4,4′-dithiobisbenzoate (**2**, 0.91 g, 3 mmol) in EtOH (30 ml) was reduced with $NaBH_4$ (0.2 g, 5.3 mmol) at room temperature. Completion of the reduction was monitored by the uv spectrum changing from λ_{max} (EtOH) 273 nm to λ_{max} (EtOH) 332 nm.

2-Amino-3-cyano-5-chloromethylpyrazine[15] (0.6 g, 3.7 mmol) was added to the above solution in portions and the resultant mixture was stirred at room temperature for 2 hr. A solid product was collected and washed with EtOH and Et_2O to give 0.85 g of 4 (90%): mp 154–156°; NMR (DMSO-d_6) δ 1.29 (t, 3 H, CH_2CH_3), 4.3 (s, 2 H, $-CH_2S-$), 4.24 (q, 2 H, $-CH_2CH_3$), 7.22 (s, 2 H, $-NH_2$), 7.32 and 7.78 (aromatic quartet, 4 H, J = 8.3 Hz), 8.29 (s, 1 H, C_6-H); uv λ_{max} (EtOH) 257 nm (ϵ 20,850), 290 (20,150), 360 (7600). Anal. ($C_{15}H_{14}N_4O_2S$) C, H, N.

2,4-Diamino-6-[(*S*-*p*-carbethoxyphenyl)thiomethyl]pteridine (6). To a mixture of 4 (1.5 g, 4.8 mmol) and guanidine hydrochloride (0.48 g, 5 mmol) in absolute EtOH (300 ml), NaOEt (0.4 g, 5 mmol) was added. The reaction mixture was heated under reflux for 20 hr and diluted with EtOH (200 ml). The mixture was clarified by filtration when hot and 30 mg of solid material was discarded. The filtrate was passed through a charcoal bed and washed with water to remove inorganic salt. The charcoal bed was triturated with hot DMF (3 × 100 ml) and the solvent was removed in vacuo. The solid residue was recrystallized from EtOH–H_2O to give 1.2 g (67%) of the desired product: dec >220°, mp 230–235°; NMR (DMSO-d_6) δ 1.28 (t, 3 H, CH_2CH_3), 4.46 (q, 2 H, CH_2CH_3), 4.52 (s, 2 H, $-CH_2S$), 7.4 and 7.94 (aromatic quartet, 4 H, J = 8.3 Hz), 8.69 (s, 1 H, C_7-H); uv λ_{max} (EtOH) 263 nm (ϵ 30,200), 280 (22,000), 375 (7000). Anal. ($C_{16}H_{16}N_6O_2S$) C, H.

2,4-Diamino-6-[(*S*-*p*-carbomethoxyphenyl)thiomethyl]-pteridine (5). A mixture of 4 and guanidine hydrochloride in methanol in the presence of $NaOCH_3$ was treated by the method described for **6**. The isolated product (48% yield) was identified as the methyl ester: mp 263–266° dec; NMR (TFA) δ 4.0 (s, 3 H, $-OCH_3$), 4.51 (s, 2 H, $-CH_2S$), 7.39 and 7.92 (aromatic quartet, 4 H, J = 8.3 Hz), 8.91 (s, 1 H, C_7-H); uv λ_{max} (EtOH) 263 nm (ϵ 29,000), 280 (21,900), 375 (7000). Anal. ($C_{15}H_{14}N_6O_2S$) C, H, N, S.

2-Amino-4-oxo(3*H*)-6-[*S*-(*p*-carboxyphenyl)thiomethyl]-pteridine (7). A suspension of **6** (1.1 g, 3 mmol) in 2 *N* NaOH–EtOH (300 ml) was heated under reflux under N_2 atmosphere for 5 hr. The resultant solution was treated with charcoal; the pH was adjusted to 3.5 with 1 *N* HCl. The precipitated product was collected by centrifugation and washed with H_2O (twice), CH_3CN, and ether to give a pure product: 0.7 g (71%); mp >360° (dec >260°); NMR (TFA) δ 4.51 (s, 2 H, $-CH_2S$), 7.38 and 7.98 (aromatic quartet, 4 H, J = 8.2 Hz), 8.9 (s, 1 H, C_7H); uv λ_{max} (0.01 *N* NaOH) 257 nm (ϵ 34,100), 280 (20,600), 365 (8700). Anal. ($C_{14}H_{11}N_5O_3S$) C, H, N.

Diethyl 10-Thiafolate (8). To a suspension of **7** (500 mg, 1.53 mmol) in DMF–DMSO (30 ml, 1:1) triethylamine (200 mg, 2 mmol) was added. The mixture was gently heated and the resultant clear solution was cooled to 0°. To the chilled solution was added isobutyl chloroformate (210 mg, 1.53 mmol) and the mixture was stirred for an additional 15 min. After adding free diethyl L-glutamate (400 mg, 2 mmol) in THF (3 ml) the mixture was stirred for 0.5 hr at room temperature. The solvent was removed by rotatory evaporation in vacuo at 50° and the residue was triturated with ether, ethyl acetate, and 95% ethanol to give a yellow solid product (430 mg) (55%), dec >265°, mp 284–286°. This product (100 mg) was dissolved in a minimum amount of DMF and diluted with 0.001 *N* NaOH (50 ml) and titrated back to pH 7 with HCl to remove any contamination by unreacted starting material **6**. Precipitated product was collected by centrifugation and washed with H_2O, CH_3CN, and ether: NMR (DMSO-d_6) δ 1.15 (m, 6 H, $-CH_2CH_3$), 4.1 (q, 4 H, $-CH_2CH_3$), 4.48 (s, 2 H, $-CH_2S$), 7.4 and 7.74 (aromatic quartet, 4 H, J = 8.3 Hz), 8.64 (s, 1 H, C_7-H); uv λ_{max} (0.01 *N* NaOH) 257 nm (ϵ 34,700), 280 (23,800), 365 (9500). Anal. ($C_{23}H_{26}N_6O_6S\cdot0.5H_2O$) C, H, N.

10-Thiafolic Acid (9). A suspension of **8** (130 mg) in 0.1 *N* NaOH (50 ml) was heated at 80° under N_2 atmosphere for 40 min. The resultant solution was made acidic (pH 2.7) at 0° and the precipitated product was collected by centrifugation and then washed with H_2O, CH_3CN, and ether, respectively: NMR (TFA) δ 2.8 [b, $HNCH(CH_2CH_2-)_2$-], 4.46 (s, 2 H, CH_2S), 7.37 and 7.67 (aromatic quartet, 4 H, J = 8.3 Hz), 8.9 (s, 1 H, C_7-H); uv λ_{max} (0.01 *N* NaOH) 257 nm (ϵ 34,000), 280 (22,700), 365 (9350). Anal. ($C_{19}H_{18}N_6O_6S\cdot0.5H_2O$) C, H, N.

Tetraethyl 4,4′-Dithiobis(*N*-benzoyl-L-glutamate) (10). To a suspension of 4,4′-dithiobis(benzoic acid) (**1**, 7.7 g, 25 mmol) and diethyl L-glutamate (10.3 g, 50 mmol) in CH_3CN (300 ml), a 10%

Table I. Microbiological Activities of Thia Derivatives[a]

Compd	*Strept. faecium* ATCC 8043	*Strept. faecium* MTX-resistant	*L. casei* ATCC 7469	*L. casei* MTX-resistant
2	>400		>400	
3	>400		>2,000	
4	>2,000		>2,000	
6	8	180	70	>2,000
5	6	180	84	>2,000
7	>400		>2,000	
8	>400		>2,000	
9	50	1,520	740	>1,000
12	9	360	13	>2,000
13	2.4	>2,000	0.01	>2,000
MTX	0.15	6,000	0.01	38,000
Aminopterin	1		0.03	

[a] Microbiological assays were carried out as described previously.[21] Concentration in ng/ml for 50% inhibition. Folate is present at 1 ng/ml concentration.

Table II. Inhibitor Concentration for 50% Inhibition of *L. casei* Dihydrofolate Reductase and *L. casei* Thymidylate Synthetase[a]

Compd	Concentration, *M*	
	DHF reductase	Thymidylate synthetase
5	3.5×10^{-6}	$>3 \times 10^{-5}$
6	3.4×10^{-6}	$>3 \times 10^{-5}$
9	5×10^{-5}	$>3 \times 10^{-5}$
12	6.0×10^{-6}	$>1.0 \times 10^{-4}$
13	1.6×10^{-8}	$>1.0 \times 10^{-4}$
MTX	3.3×10^{-9}	$>3 \times 10^{-5}$

[a] Assays were carried out as described previously.[22]

excess of DCC (11.9 g, 55 mmol) was added. The reaction mixture was stirred under anhydrous conditions at room temperature for 3 days. After removal of dicyclohexylurea by filtration the solvent was evaporated from the filtrate. The oily residue was dissolved in $CHCl_3$ (350 ml) and washed with 10% $NaHCO_3$ (2 × 50 ml), 0.1 *N* HCl (2 × 50 ml), and water. The organic layer was dried ($MgSO_4$) and the solvent removed. The oily residue was redissolved in warm CH_3CN (350 ml) and CH_3CN-insoluble material was discarded. Concentration of the filtrate followed by cooling gave 7.9 g (46%) of the desired colorless, crystalline product: mp 97–99°; NMR ($CDCl_3$) δ 1.23 (m, 6 H, $-CH_2CH_3$), 4.08 (q, 4 H, $-CH_2CH_3$), 7.44 and 7.71 (aromatic quartet, 4 H, J = 8.3 Hz); uv λ_{max} (EtOH) 270 nm (ϵ 31,200). Anal. ($C_{32}H_{40}N_2O_{10}S_2$) C, H, N.

Diethyl *N*-[*p*-[(2-Amino-3-cyanopyrazin-5-yl)methylthio]benzoyl]-L-glutamate (11). Compound **10** (3.38 g, 5 mmol) in absolute EtOH (100 ml) was reduced with $NaBH_4$ (0.37 g, 10 mmol) at room temperature. When the reduction was completed, 2-amino-3-cyano-5-chloromethylpyrazine[15] (1.68 g, 10 mmol) was added in small portions within 5 min. The mixture was stirred at room temperature for 2.5 hr. On TLC the fluorescent spot of 2-amino-3-cyano-5-chloromethylpyrazine was completely replaced by a quenching spot [R_f 0.48; $CHCl_3$–MeOH (19:1)] and the sodium mercaptide spot (origin) disappeared, and two new minor spots (R_f 0.7 and 0.3) appeared. The solvent was removed in vacuo, the residue was suspended in water (150 ml) and extracted with $CHCl_3$ (3 × 50 ml). The extract was dried ($MgSO_4$) and the solvent removed. Development with ether of preparative layer chromatograms (silica gel PF 254, E. M. Reagents Div., Brinkmann) separated the three components. The major component (R_f 0.48) was identified as the desired product, a very thick oil, 2.2 g (47%). The minor by-products were not identified. Column chromatography with Florisil was not successful: NMR ($CDCl_3$) δ 1.25 (m, 6 H, $-CH_2CH_3$), 4.16 (q, 4 H, $-CH_2CH_3$), 4.14 (s, 2 H, $-CH_2S$), 7.2 and 7.65 (aromatic quartet, 4 H, J = 8.3 Hz), 8.11 (s, 1 H, C_6-H).

Diethyl 10-Thiaaminopterin (12). To a mixture of the pyrazine **11** (1.24 g, 2.5 mmol) and guanidine hydrochloride (0.24 g, 2.5 mmol) in EtOH (150 ml) was added NaOEt (0.135 g, 2.5 mmol). The reaction mixture was heated under reflux for 16 hr and the mixture was passed through a charcoal–Celite bed. After removal of the solvent, the semisolid residue was thoroughly triturated with water to remove any inorganic salt to give 0.75 g (58%) of solid product. Recrystallization from CH_3CN (twice) gave an analytically pure, yellow crystalline product: mp 192–194°; NMR (DMSO-d_6) δ 1.15 (m, 6 H, $-CH_2CH_3$), 4.04 (q, 4 H, $-CH_2CH_3$), 7.41 and 7.63 (aromatic quartet, 4 H, J = 8.3 Hz), 8.68 (s, 1 H, C_7-H); uv λ_{max} (EtOH) 263 nm (ϵ 31,400), 285 (21,500), 375 (8350). Anal. ($C_{23}H_{27}N_7O_5S$) C, H, N.

10-Thiaaminopterin (13). A suspension of **12** (100 mg) in 0.02 *N* NaOH–CH_3CN (3:1, 40 ml) was stirred under N_2 atmosphere at room temperature; the mixture became homogeneous within 1 hr. The stirring was continued for an additional 30 min. After completion of the saponification, which was confirmed by TLC ($CHCl_3$–MeOH, 19:1), CH_3CN was removed in vacuo and the residue diluted with water (300 ml). Water-insoluble material was removed by filtration (Celite) and the filtrate carefully adjusted to pH 3 with 0.1 *N* HCl. Precipitated product was collected by centrifugation and washed with water (twice) and CH_3CN, respectively, to obtain 90 mg of beige product (96%): dec >180°, mp 239–241°; NMR (TFA, TMS external standard) δ 4.25 (s, 2 H, $-CH_2S$), 7.15 and 7.45 (aromatic quartet, 4 H, J = 8.3 Hz), 8.68 (s, 1 H, C_7-H); uv λ_{max} (0.01 *N* NaOH) 260 nm (ϵ 33,000), 280 (22,000), 373 (8500). Anal. ($C_{19}H_{19}N_7O_5S$) C, H, N, S.

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Synthesis and Biological Activities of Some N^6-(Nitro- and -aminobenzyl)adenosines†

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Synthesis and biological activities of 12 analogs of N^6-benzyladenosine are described. The compounds were prepared by two methods: (1) direct alkylation of adenosine with an appropriately substituted benzyl bromide to give the N^1-substituted derivative which was then rearranged in base to give the N^6-substituted compound, and (2) by nucleophilic displacement of chlorine in 6-chloropurine ribonucleoside, 6-chloro-2-aminopurine ribonucleoside, and 6-chloro-2-aminopurine with an amine. These analogs were examined for their growth inhibitory effect in cultured leukemic cells and also for their effect on adenosine aminohydrolase activity. N^6-*p*-Nitrobenzyladenosine and its 2′-deoxy analog were competitive inhibitors (K_i 65, 22 μM). The 2-amino-N^6-*p*-nitrobenzyladenine and its ribonucleoside were found to be noncompetitive inhibitors of adenosine aminohydrolase. In cultured L1210 leukemia, 2-amino-6-*p*-nitrobenzylaminopurine and the corresponding ribonucleoside were better growth inhibitors than N^6-benzyladenosine, while N^6-*p*-nitrobenzyladenosine, its 2′-deoxy analog, and N^6-*p*-fluorobenzyladenosine were as active as N^6-benzyladenosine.

The clinical usefulness of N^6-benzyladenosine in neoplastic disease[1] prompted the synthesis and exploration of the biological activity of nitro and aminobenzyl analogs of this compound. N^6-Benzyladenosine has been shown to possess growth inhibitory activity in tumor cell systems in vitro and in vivo.[2,3] Some of the N^6-benzyladenosine derivatives have exhibited inhibitory activity toward the uptake of purines and pyrimidines in cultured cells.[4,5] It stimulates the growth of tobacco pith tissue in vitro[6] and in cell-free systems acts as a competitive inhibitor of adenosine aminohydrolase.[7] Reported herein are the syntheses of 12 analogs of N^6-benzyladenosine, their growth inhibitory properties in three cell lines in culture, and their effect on adenosine aminohydrolase activity.

Chemistry. N^6-*p*-Nitrobenzyladenosine (**2**) was synthesized by a reaction of adenosine with *p*-nitrobenzyl bromide in DMF to furnish the N^1-*p*-nitrobenzyladenosine derivative. The latter was rearranged in base to give N^6-*p*-nitrobenzyladenosine.[8] Similarly *o*- and *m*-nitrobenzyladenosine (**3** and **4**) and *p*-fluorobenzyladenosine (**9**) were prepared starting from adenosine and an appropriately substituted benzyl bromide. 2′-Deoxyadenosine was used as the starting material for the preparation of 2′-deoxy-N^6-benzyladenosine (**13**)[9] and 2′-deoxy-N^6-*p*-nitrobenzyladenosine (**5**). Catalytic reduction of N^6-*p*-nitrobenzyladenosine gave N^6-*p*-aminobenzyladenosine (**7**), which was isolated as the HCl salt. Acetylation of the latter afforded N^6-*p*-acetamidobenzyladenosine (**8**). Reaction of methyl isocyanate with 2′,3′,5′-tri-*O*-acetyl-N^6-benzyladenosine followed by deacetylation gave a more soluble compound, *N*-[(9-β-D-ribofuranosylpurin-6-yl)-*N*-benzylcarbamoyl]methylamine (**10**). Treatment of N^6-benzyladenosine (**1**) with methanolic hydrogen chloride gave the N^6-benzyladenosine hydrochloride (**14**), which was more soluble in water than the parent compound. However, it was less stable. Nucleophilic displacement of chlorine in 6-chloro-2-aminopurine and 6-chloro-2-aminopurine ribonucleoside with *p*-nitrobenzylamine[10,11] gave 2-amino-6-*p*-nitrobenzylaminopurine (**12**) and 2-amino-6-*p*-nitrobenzylaminopurine ribonucleoside (**11**), respectively. N^6-(2-Methoxy-5-nitro)phenyladenosine (**6**) was similarly prepared starting from 6-chloropurine ribonucleoside and 2-methoxy-5-nitroaniline. The structures of all these compounds were confirmed by their uv, ir, and NMR spectra as well as from their correct elemental analyses. The physical properties of these analogs are listed in Table I.

Experimental Section

Melting points were determined by a Mel-Temp laboratory model melting point apparatus and are uncorrected. Uv spectra were recorded on a Cary 14 and Beckman Acta-V spectrophotometer. NMR spectra were recorded on a Varian A-60A spectrometer in DMSO-d_6 solution using Me_4Si as an internal standard. Elemental analyses were carried out by Heterocyclic Chemical Corporation, Harrisonville, Mo. Homogeneity of all the compounds was checked by thin-layer chromatography in three solvent systems: (a) *i*-PrOH–concentrated NH_4OH–H_2O (7:1:2 v/v); (b) EtOAc–2-ethoxyethanol–16% HCO_2H (4:1:2 v/v, upper phase); and (c) EtOAc–*n*-PrOH–H_2O (4:1:2 v/v, upper phase).

N^6-*p*-Nitrobenzyladenosine (2). Method A. To a solution of 1.33 g of adenosine (5.0 mmol) in 25.0 ml of freshly distilled anhydrous DMF was added 3.24 g (15.0 mmol) of *p*-nitrobenzyl bromide and the mixture stirred in a stoppered flask at room temperature for 48 hr. The reaction mixture was then evaporated to dryness in vacuo at 45°. The gummy residue was triturated three times with 50 ml of hot acetone. The crude residue was dissolved in 50 ml of distilled water and the pH of the solution was adjusted to 9.0–9.5 by careful addition of diluted aqueous NH_4OH. The mixture was then heated on a steam bath for 3 hr. The pH of the solution was maintained within the range of 9.0–9.5 by addition of diluted aqueous NH_4OH from time to time. The reaction mixture was evaporated to a small volume and kept at 4° for 20 hr. A yellow crystalline product was collected on a filter and washed with H_2O. Two recrystallizations from absolute EtOH furnished 1.25 g (62.2%) of pure product: mp 174–176°; NMR δ 8.53 (s, 1, 8-H), 8.33 (s, 1, 2-H), 8.27 and 7.67 (pair of doublets, 4, J = 8 Hz in each case, protons of phenyl ring), 5.98 (d, 1, J = 6 Hz, 1′-H), 5.43 (d, 2, J = 7 Hz, $-CH_2C_6H_5$). Anal. ($C_{17}H_{18}N_6O_6$) C, H, N.

N^6-*p*-Aminobenzyladenosine Hydrochloride (7). To a solution of compound **2** (500 mg) in 125 ml of MeOH was added 500

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