Synthesis and Biological Evaluation of 9-Thia-5,10-dideazafolic Acid†

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The folate analogue, 9-thia-5,10-dideazafolic acid (**3b**), was obtained in an efficient two-step procedure in an overall yield of 60%. The previously unknown intermediate dimethyl-thiocarbamic acid S-(2-amino-3,4-dihydo-4-oxo-pyrido[2,3-*d*]pyrimidin-6-yl) ester (**5**) was prepared *via* the condensation of 2,6-diamino-3*H*-pyrimidin-4-one and S-(2-malonaldehyde)-1,1,3,3-tetramethylthiouronium bromide (**4**). Compound **5**, in a one pot procedure, was deprotected using sodium hydroxide and then coupled to diethyl *N*-[(4-chloromethyl)benzoyl]-L-glutamate, followed by saponification of the ethyl esters to give the 9-thia-5,10-dideazafolic acid (**3b**). Compound **3b** was a potent inhibitor of human 5-aminoimidazole-4-carboxamide ribonucleotide transformylase (K_i of 8 ± 5 μM) and showed no inhibition of human glycinamide ribonucleotide transformylase at concentrations as high as 50 μM. Compound **3b** was screened by the National Cancer Institute Developmental Therapeutics Program against 60 human tumors and was found to be active against a leukemia RPMI-8226 cell line where the LC_{50} was 1 μM.

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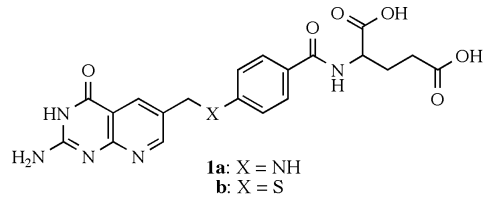
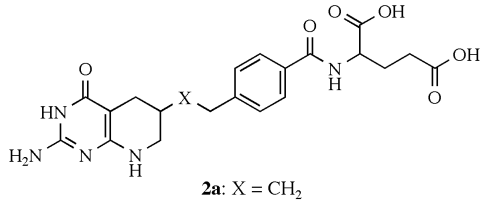
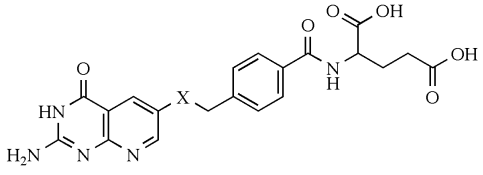
The discovery that folic acid analogs such as methotrexate are useful therapeutic agents in the treatment of neoplastic and inflammatory diseases has prompted the synthesis of numerous folate derivatives. Of particular interest are derivatives of 5-deazafolic acid (**1a**), which have shown antitumor activity *via* the selective inhibition of glycinamide ribonucleotide transformylase (GAR Tfase), the first of two enzymes requiring folate in *de novo* purine biosynthesis. One of the more promising of these analogs, 5,10-dideaza-5,6,7,8-tetrahydrofolic acid (lometrexol) (**2a**), showed broad spectrum antitumor activity, was active against methotrexate resistant cell lines and is now in clinical trials [1-3]. Other analogs that have been prepared in this series are 10-thia-5-deazafolic acid (**1b**) [4], and N^9-substituted analogs of 5-deazaisofolic acid (**3a**) and 5-deaza-5,6,7,8-tetrahydroisofolic acid (**2b**) [5]. The most potent of these compounds (**2b**) had an IC_{50} of 1 μM against MCF-7 cells, which is 100 fold less potent than **2a**. In an effort to further explore the therapeutic potential of 5-deaza analogues of folic acid we have prepared and determined the biological properties of 9-thia-5,10-dideazafolic acid (**3b**).



1a: X = NH
b: X = S



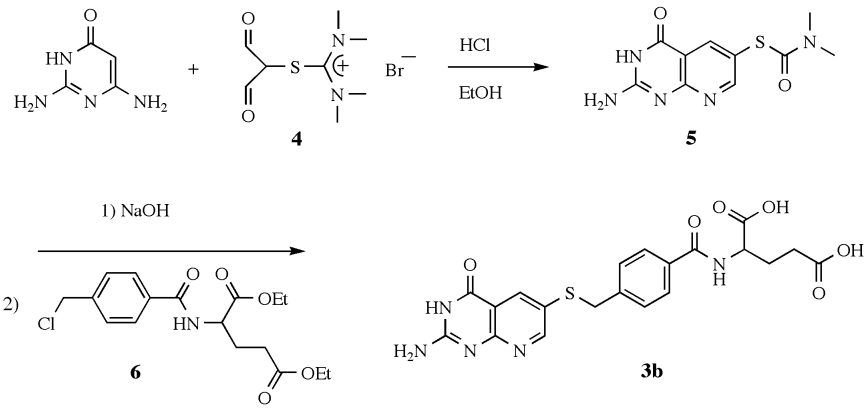
2a: X = CH_2
b: X = NH



3a: X = NH
b: X = S

The synthetic route used to prepare 9-thia-5,10-dideaza folic acid is shown in Scheme 1. The compound was prepared in a very efficient manner from readily available intermediates in two steps in overall yield of 60%. The key starting material, S-(2-malonaldehyde)-1,1,3,3-tetramethylthiouronium bromide (**4**), obtained from the reaction of 1,1,3,3-tetramethylthiourea and 2-bromomalonaldehyde, crystallized from solution as the analytically pure bromide salt. The use of tetramethylthiourea was necessary in order to prevent intramolecular cyclization to the thiazole [6]. The synthesis of 6-substituted-5-deazapurines *via* the condensation of 2,6-diamino-3*H*-pyrimidin-4-one with malonaldehydes has been reported for 2-nitromalonaldehyde [7], triformylmethane (2-formylmalonaldehyde) [8] and 2-bromomalonaldehyde [9]. In a similar manner malonaldehyde **4** condensed readily with 2,6-diamino-3*H*-pyrimidin-4-one under acidic conditions to give compound **5** in 85% yield. This is the first example of a thiomalonaldehyde equivalent in this reaction, which under aqueous acid conditions the tetramethylthiouronium group is hydrolyzed to the *N*,*N*-dimethylcarbamate protected thiol. Compound **5** was very insoluble and we were not able to find a suitable solvent for recrystallization, however, the compound was of sufficient purity for use in the next step. In a one-pot procedure, the dimethylcarbamate group of **5** was first hydrolyzed in refluxing 1 *N* sodium hydroxide to give the sulfide, which was then

Scheme 1



Synthetic Route to 9-Thia-5,10-dideazafolic Acid.

coupled to **6**, followed by saponification of the ethyl esters and acidification of the reaction mixture to give the target compound, 10-thia-5,10-dideazafolic acid (**3b**), in 73% yield. Attempts to reduce the ring using catalytic hydrogenation over platinum oxide or 10 % palladium on carbon were unsuccessful.

The compound (**3b**) was assayed for inhibition of both folate requiring enzymes in *de novo* purine biosynthesis: GAR Tfase and 5-aminoimidazole-4-carboxamide ribonucleotide transformylase (AICAR Tfase). Compound **3b** was a potent inhibitor of human AICAR Tfase (K_i of 8 ± 5 μM) with an affinity 5 times greater than its cofactor 10-formyltetrahydrofolate [10]. However, there was no inhibition of human GAR Tfase at concentrations as high as 50 μM. Compound **3b** was screened by the National Cancer Institute Developmental Therapeutics Program against 60 human tumor cell lines. The compound was found to be most active against a leukemia RPMI-8226 cell line where the LC_{50} was 1 μM.

EXPERIMENTAL

Melting points were taken on a Mel-Temp II in an open capillary and are uncorrected. The ^1H and ^{13}C NMR spectra were taken on a Bruker AMX-360 spectrophotometer, MALDI and APCI mass spectra were obtained on a Perseptive Biosystems (Framingham, MA), and FAB mass spectra on a Kratos Analytical (Manchester, UK). Elemental analysis was performed by Atlantic Microlab, Inc., Norcross, Georgia.

S-(2-Malonaldehyde)-1,1,3,3-tetramethylthiouronium Bromide (**4**).

To a solution of 1.5 g (10 mmol) of 2-bromomalonaldehyde [11] in 20 mL of acetone at 25 °C was added 1.3 g (10 mmol) of 1,1,3,3-tetramethylthiourea. The solution was stirred at 25 °C and after approximately 5 minutes a precipitate was observed. Stirring was continued for an additional 30 minutes and the white precipitate was collected by filtration and washed with acetone to give 2.7 g (96%) of **4**, mp 147-148 °C; ^1H NMR (D_2O): δ 8.66 (s, 2H), 2.97 (s, 12H); ^{13}C NMR (D_2O): δ 186.6, 176.4, 107.2, 43.4; MALDI ms: m/z 203 (M^+).

Anal. Calcd. for $C_8H_{15}N_2O_2SBr$: C, 33.93; H, 5.34; N, 9.89. Found: C, 34.14; H, 5.48; N, 9.66.

Dimethyl-thiocarbamic Acid S-(2-Amino-3,4-dihydo-4-oxo-pyrido[2,3-d]pyrimidin-6-yl) Ester (**5**).

A mixture of 0.6 g of (4.7 mmol) of 2,6-diamino-3*H*-pyrimidin-4-one and 1.4 g (4.9 mmol) of S-(2-malonaldehyde)-1,1,3,3-tetramethylthiouronium bromide in 30 mL of ethanol and 5 mL of 12 *N* hydrochloric acid was refluxed for 1 hour. The solution was cooled to 25 °C, concentrated and the residue triturated with 5% sodium hydrogencarbonate. The precipitate was collected by filtration, washed with water and ethanol, and dried under vacuum at 60 °C to give 1.1 g (85 %) of a yellow-brown solid, mp >250 °C. This compound could not be purified and fully characterized due to its insolubility, however it was of sufficient purity for use in the preparation of **3b**, ^1H NMR (DMSO): δ 11.61 (br s, 1H, N-H), 8.51 (d, 1H, H-7, $J_{7,5}$ = 2.5 Hz), 8.15 (d, 1H, H-5, $J_{5,7}$ = 2.5 Hz), 7.08 (br s, 2H, NH_2), 3.07 (s, 3H), 2.94 (s, 3H); ^{13}C (d-TFA): δ 169.98, 162.13, 157.28, 156.68, 154.44, 152.67, 125.96, 116.28, 39.23, 38.78; FAB ms: m/z 266 (MH^+).

Diethyl *N*-[(4-Chloromethyl)benzoyl]-L-glutamate (**6**).

This compound was prepared in a manner similar to that for the preparation of diethyl *N*-[(4-bromomethyl)benzoyl]-L-glutamate [12]. To a solution of 0.9 g (4.8 mmol) of 4-(chloromethyl)benzoyl chloride in 2 mL of dichloromethane, cooled to 0 °C, was added dropwise a solution of 1.2 g (5.0 mmol) of diethyl L-glutamate hydrochloride and 1.4 mL (10 mmol) of triethylamine in 3 mL of dichloromethane. The mixture was stirred for 1 hour at 0 °C and then 2 hours at 25 °C. The solution was diluted with dichloromethane and washed with 0.1 *N* hydrochloric acid and then brine. The solution was dried (sodium sulfate) and evaporated to give 1.5 g (85%) of a white solid, mp 110 °C; ^1H NMR ($CDCl_3$): δ 7.80 (d, 2H, 2',6', J_o = 8.2 Hz), 7.45 (d, 2H, 3',5', J_o = 8.2 Hz), 7.05 (d, 1H, NH, J = 9.8 Hz), 4.76 (m, 1H, glutamate α H), 4.59 (s, 2H, CH_2Cl), 4.22 (m, 2H, -OCH_2CH$_3$), 4.09 (m, 2H, -OCH_2CH$_3$), 2.10-2.58 (m, 4H, glutamate β and γ H's), 1.28 (t, 3H, -OCH$_2$CH_3 J = 7.3 Hz), 1.20 (t, 3H, -OCH$_2$CH_3,J = 7.3 Hz); ^{13}C NMR ($CDCl_3$): δ 173.27, 171.87, 166.43, 141.05, 133.56,

128.66, 127.52, 61.71, 60.81, 52.40, 45.31, 30.44, 27.05, 14.11, 14.08; APCI ms: m/z 356 (MH^+).

Anal. Calcd. for $C_{17}H_{22}NO_5Cl$: C, 57.38; H, 6.23; N, 3.94. Found: C, 57.23; H, 6.15; N, 4.05.

9-Thia-5,10-dideazafolic Acid (**3b**).

A solution of 0.27 g (1.0 mmol) of **5** in 2.8 mL of degassed 1 *N* sodium hydroxide was refluxed for 1.5 hours. The solution was cooled to 25 °C and a solution of 0.39 g (1.1 mmol) of diethyl *N*-[(4-chloromethyl)benzoyl]-L-glutamate in 1.5 mL of ethanol was added. The reaction mixture was stirred for 20 minutes at 25 °C and then 1.5 mL of 1 *N* sodium hydroxide was added and stirring continued for 10 hours. The pH was adjusted to 3 with 6 *N* hydrochloric acid and the resulting precipitate was collected by filtration and washed with water, ethanol, and diethyl ether and dried under vacuum at 60 °C to give 0.33 g (73 %) of **3b**, mp >250 °C; uv: (pH 7.5): λ max 285 nm (ε 17,600), 333 nm (ε 4,700); ^{1}H NMR (0.1 *N* NaOD): δ 8.31 (d, 1H, H-7, $J_{7,5}$ = 2.3 Hz), 8.28 (d, 1H, H-5, $J_{5,7}$ = 2.3 Hz), 7.70 (d, 2H, J = 6.2 Hz), 7.28 (d, 2H, J = 6.2 Hz), 4.33 (dd, 1H, J = 9.0 Hz, J = 4.5 Hz), 4.16 (s, 2H), 2.32 (dd, 2H, J = 7.9 Hz, J = 7.9 Hz), 2.12 (m, 2H); ^{13}C NMR (0.1 *N* NaOD): δ 182.66, 179.26, 174.61, 171.39, 160.97, 158.32, 142.27, 141.71, 132.70, 129.58, 127.73, 122.25, 112.28, 56.35, 40.37, 34.62, 28.73; MALDI ms: m/z 458 (MH^+).

Anal. Calcd. for $C_{20}H_{19}N_5O_6S\bullet 1.3H_2O$: C, 49.95; H, 4.53; N, 14.56. Found: C, 50.19; H, 4.56; N, 14.26.

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