Synthesis of Selected 3-Substituted-Pyrimido[5,4-*e*]-1,2,4-triazine-5,7-diamines as Potential Folate Antagonists [1,2]

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Received October 22, 1984

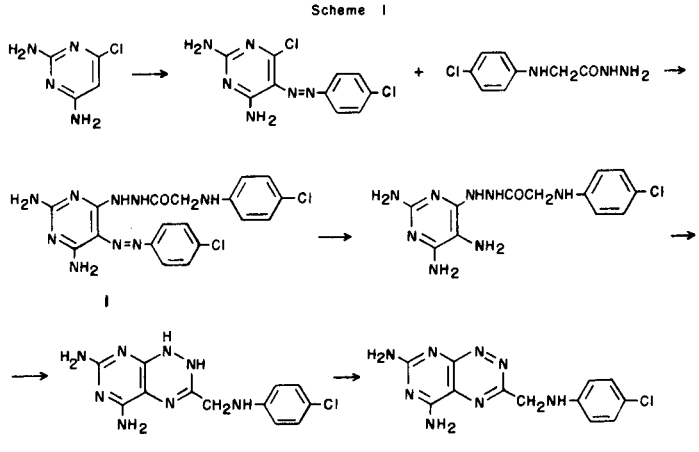
3-(Anilinomethyl) and 3-[(arylthio)methyl]pyrimido[5,4-*e*]-1,2,4-triazine-5,7-diamines were prepared by ring closure of 6-hydrazino-4-[(phenylmethyl)thio]-2,5-pyrimidinediamine. These nonclassical analogs of known potent dihydrofolate reductase inhibitors were inactive against malarial infections in mice and L1210 leukemia *in vitro*.

J. Heterocyclic Chem., **22**, 1369 (1985).

Our interest in the potent antiparasitic and anticancer [3] activity of a variety of nonclassical 2,4-diaminoquinazolines and related structures made it of interest to examine the biological properties of the analogous pyrimido-[5,4-*e*]-1,2,4-triazines. Derivatives of this ring system are much less readily available and thus have been investigated only minimally. The synthesis and preliminary biological evaluation of selected analogs of this ring system are the subject of this report.

Initially we investigated a route (Scheme I) involving closure of the triazine ring on to a preformed diamino pyrimidine.

Scheme I



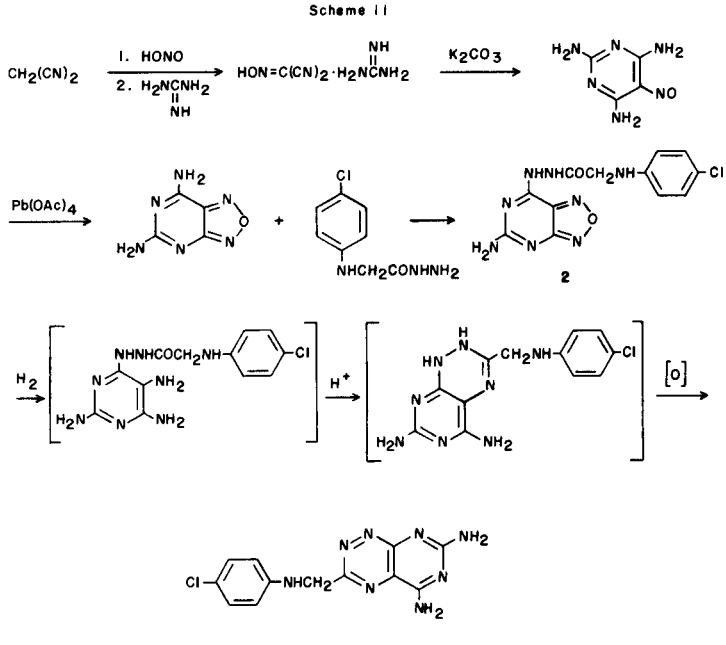
This scheme was successful up to the azo dye **1**, however we were unable to reduce this to the amine in any kind of useful yield.

We then attempted to utilize a modification of a route developed by Taylor [4] (Scheme II).

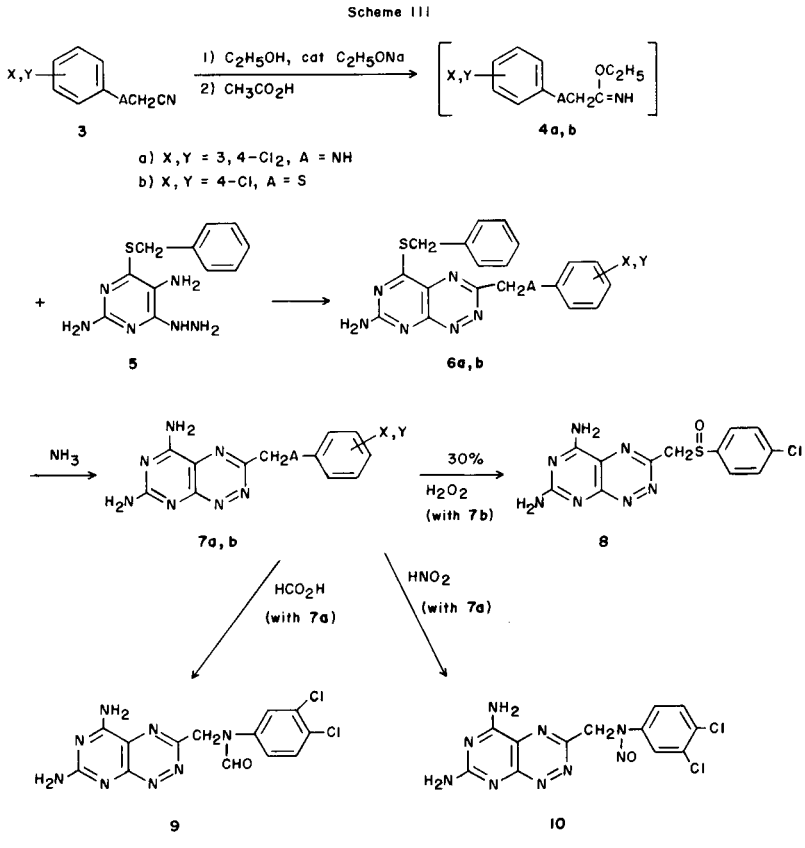
This route has been carried through successfully to the *N*-(4-chlorophenyl)glycine, 2-(5-amino-[1,2,5]oxadiazolo-[3,4-*d*]pyrimidin-7-yl)hydrazide (**2**). However reductive cleavage of this material presented considerable difficulty and was ultimately abandoned.

The 3-substituted pyrimido[5,4-*e*]-1,2,4-triazine-5,7-diamines **7a,b** were finally prepared following an approach

Scheme II



Scheme III



developed by Temple and associates [5] (Scheme III). Thus [(3,4-dichlorophenyl)amino]acetonitrile (**3a**) [6] was treated with ethanol in the presence of a catalytic amount of sodium ethoxide to form 2-[(3,4-dichlorophenyl)amino]ethanimidic acid, ethyl ester (**4a**) [7] which was not isolated but allowed to condense with 4-hydrazino-6-[(phenylmethyl)-thio]-2,5-pyrimidinediamine (**5**) [5] to give the 5-[(phenylmethyl)thio]pyrimido[5,4-*e*]-1,2,4-triazine (**6a**). Treatment of **6a** with ammonia afforded the desired pyrimido[5,4-*e*]-1,2,4-triazine-5,7-diamine (**7a**). The 3-[[(4-chlorophenyl)-thio]methyl]pyrimido[5,4-*e*]-1,2,4-triazine-5,7-diamine (**7b**) was prepared analogously from [(4-chlorophenyl)thio]acetonitrile. The sulfinyl derivative **8** was prepared by treatment of **7b** with 30% hydrogen peroxide in acetic acid. Treatment of **7a** with formic acid and with nitrous acid afforded the formamide **9** and nitroso **10** derivatives, respectively.

The pyrimido[5,4-*e*]-1,2,4-triazines, **7a, 7b, 8-10**, were administered subcutaneously in a single dose to mice infected with a normal drug-sensitive strain of *Plasmodium berghei* [8] and were found devoid of antimalarial activity at 640 mg/kg, the highest dose tested. Compounds **7a, 7b, 9, 10** were also tested *in vitro* against *Streptococcus faecalis* (MGM-2), normal (UC-76), and drug-resistant (S18713) *Staphylococcus aureus, Pseudomonas aeruginosa* (28), *Escherichia coli* (Vogel), *Shigella sonnei* (C-10), and *Mycobacterium tuberculosis* $H_{37}Rv$ using a modification of the gradient procedure of Szybalski [9] and Webb and Washington [10]. Inhibition of *Mycobacterium tuberculosis* $H_{37}Rv$ at 10 μg/ml by **7a** and **9** was the only activity demonstrated.

In addition, compounds **7a, 7b, 9, 10** were evaluated against the L1210 leukemia in tissue culture [11] and were inactive at the highest dose given, 1 μg/ml.

Substitution of carbon by nitrogen in the carbocyclic ring of 2,4-diaminoquinazolines once again appears not to be a fertile modification for improved biological activity. Although electronic considerations leading to decreased binding capability to an active site in a key enzyme may be invoked to explain such lowering of activity, more detailed studies must be performed to allow explanation of this phenomenon.

EXPERIMENTAL

Melting points (corrected) were taken on a Thomas-Hoover capillary melting point apparatus. The ir (potassium bromide) and nmr (deuterated dimethylsulfone) spectra were obtained on all compounds cited and were consistent with the assigned structures.

N-(4-Chlorophenyl)glycine, 2-[2,6-Diamino-5-[(4-chlorophenyl)azo-4-pyrimidinyl)hydrazide, Hemihydrate (**1**).

A solution of 2.83 g (0.01 mole) of 6-chloro-5-[(4-chlorophenyl)azo]-2,4-pyrimidinediamine [12] and 2.00 g (0.01 mole) of *N*-(4-chlorophenyl)glycine hydrazide [13] in 175 ml of dioxane was heated under reflux. After 20 hours, the hot mixture was filtered and the solid was dried *in vacuo* to give 3.65 g of crude product as the hydrochloride salt, mp 281-282°. One g of the solid was dissolved in 15 ml of dimethylsulfoxide to which 2 ml of concentrated ammonium hydroxide had been added, and the solution was filtered through supercell. The free base was precipitated by the addition of 115 ml of water, collected by filtration, and dried *in vacuo* to give 0.76 g , mp 142-149°. This was recrystallized from a mixture of dioxane and water to give 0.38 g of the title compound, mp 217-219°.

Anal. Calcd. for $C_{18}H_{17}Cl_2N_9O{\cdot}0.5H_2O$: C, 47.48; H, 3.88; N, 27.69; H_2O, 1.98. Found: C, 47.22; H, 3.85; N, 27.66; H_2O, 2.13.

The remaining 2.10 g of hydrochloride was recrystallized from a mixture of dioxane and water which had been made strongly basic with ammonium hydroxide to give another 1.45 g of material, mp 224-225°. The total yield of 1.83 g of free base represents a yield of 40%.

Guanidine Salt of 2-(Hydroxyimino)propanedinitrile.

A solution of 55.0 g (0.796 mole) of sodium nitrite in 80 ml of water was added dropwise over 3 hours to a stirred solution of 50.0 g (0.757 mole) of propanedinitrile in a mixture of 47.5 ml of glacial acetic acid and 40 ml of water maintained at 6° in an ice bath. The mixture was allowed to warm to room temperature and then allowed to stand for 40 hours. Stirring was resumed and the mixture was heated to 50° while 75.0 g (0.414 mole, 0.828 equivalent) of guanidine carbonate was added in small portions. When addition was complete, stirring was discontinued and the mixture was cooled to 0° in an ice-salt bath. The mixture was filtered, the filter cake was dissolved in 250 ml of warm (50°) water, cooled overnight in the refrigerator to 10°, and then cooled in an ice-salt bath to 0°. The solid was collected and dried in air to give 40.8 g (35%) of the product.

5-Nitroso-2,4,6-pyrimidinetriamine.

To a solution of 42.8 g (0.279 mole) of the guanidine salt of 2-(hydroxyimino)propanedinitrile in 185 ml of *N,N*-dimethylformamide was added 98 g (0.0713 mole) of potassium carbonate and the mixture was heated under reflux with stirring for 0.5 hours. The mixture slowly became purple. After cooling slightly 250 ml of water was added and the mixture filtered. The deep pink solid was stirred vigorously with 125 ml of water and collected. The solid was washed with a mixture of 50 ml of methanol and 50 ml of ethyl ether and dried to give 30.9 g (72%) of the product, mp 345-347° dec.

[1,2,5]Oxadiazolo[3,4-*d*]pyrimidine-5,7-diamine.

To a suspension of 5-nitroso-2,4,6-pyrimidinetriamine in 150 ml of glacial acetic acid under nitrogen was added in portions over 30 minutes 30.3 g (0.0683 mole) of lead tetraacetate. The mixture was allowed to stir overnight and the solid was collected and washed with water. Recrystallization from a mixture of *N,N*-dimethylformamide-water (charcoal) gave 5.6 g (57%) of the product as yellow crystals, mp > 340°.

Anal. Calcd. for $C_4H_4N_6O$: C, 31.58; H, 2.65; N, 55.25. Found: C, 31.19; H, 2.82; N, 55.42.

N-(4-Chlorophenyl)glycine, 2-(5-Amino[1,2,5]oxadiazolo[3,4-*d*]pyrimidin-7-yl)hydrazide (**2**).

A mixture of 5 g (0.0329 mole) of [1,2,5]oxadiazolo[3,4-*d*]pyrimidine-5,7-diamine and 7.2 g (0.036 mole) of *N*-(4-chlorophenyl)glycine hydrazide [13] in 50 ml of 1 *N* ethanolic hydrogen chloride and 150 ml of ethanol was heated under reflux for 2 hours. The mixture was cooled, made basic with triethylamine and the solid was collected, washed with methanol and then with ether to give 5.75 g of light yellow solid. This material was dissolved in 25 ml of hot *N,N*-dimethylformamide and the solution was cooled. The solid that formed was removed by filtration (1.6 g, mp > 350°) and discarded. The filtrate was poured into 100 ml of warm methanol and the solid that formed was collected. Washing with methanol and then with ether gave 3.8 g (33%) of light yellow solid, mp 271° dec.

Anal. Calcd. for $C_{12}H_{11}ClN_8O_2$: C, 43.06; H, 3.31; N, 33.48. Found: C, 42.90; H, 3.39; N, 33.99.

7-Amino-*N*-(3,4-dichlorophenyl)-5-[(phenylmethyl)thio]pyrimido[5,4-*e*]-1,2,4-triazine-3-methanamine (**6a**).

A solution of 5.84 g (0.029 mole) of [(3,4-dichlorophenyl)amino]acetonitrile (**3a**) [6], and 0.09 g (0.0039 mole) of sodium in 50 ml of ethanol was stirred overnight at room temperature and neutralized with glacial acetic acid. This solution of 2-[(3,4-dichlorophenyl)amino]ethanimidic acid, ethyl ester (**4a**) was combined with a solution of 6.5 g (0.029 mole) of 4-hydrazino-6-[(phenylmethyl)thio]-2,5-pyrimidinediamine (**5**) [5] in 83 ml of dioxane. The reaction mixture was stirred at room temperature for 24 hours and then chilled. The orange precipitate that formed was collected and washed with a little ethanol to give 5.65 g (51%) of **7a**, mp 255° dec. The filtrate afforded an additional 1.43 g (total yield, 64%), mp 251-252° dec.

Anal. Calcd. for $C_{19}H_{15}Cl_2N_7S{\cdot}0.33H_2O$: C, 50.73; H, 3.49; N, 21.80; S, 7.13; Cl, 15.77. Found: C, 50.68; H, 3.75; S, 7.49; Cl, 16.07.

A correct water analysis could not be obtained, however, the nmr spectrum demonstrated the presence of approximately one-third mole of water.

3-[[(3,4-Dichlorophenyl)amino]methyl]pyrimido[5,4-*e*]-1,2,4-triazine-5,7-diamine, Hydrate (1:0.4) (**7a**).

To a hot (60°) solution of 3.94 g (0.009 mole) of 7-amino-*N*-(3,4-dichlorophenyl)-5-[(phenylmethyl)thio]pyrimido[5,4-*e*]-1,2,4-triazine-3-methanamine (**6a**) in 210 ml of *N,N*-dimethylacetamide was added a solution of 12 g (0.8 mole) of ammonia in 200 ml of ethanol. The mixture was stirred at 60° for 7 hours, chilled overnight and filtered. The filter cake was washed with ethanol and dried at 100° *in vacuo* to afford 2.35 g (77%) of **7a**, mp > 300°.

Anal. Calcd. for $C_{12}H_{10}Cl_2N_8{\cdot}0.4H_2O$: C, 41.85; H, 3.16; N, 32.54; Cl, 20.59. Found: C, 42.00; H, 3.19; N, 32.52; Cl, 20.36.

Again although a correct water analysis could not be obtained, the nmr spectrum demonstrated the presence of about 0.4 mole of water. The methylene group appears as a doublet at 5.02 ppm and as a singlet when washed with deuterated water.

3-[[(4-Chlorophenyl)thio]methyl]-5-[(phenylmethyl)thio]pyrimido[5,4-*e*]-1,2,4-triazin-7-amine (**6b**).

A solution of 0.13 g (0.006 mole) of sodium in 10 ml of ethanol was combined with a solution of 8 g (0.044 mole) of [(4-chlorophenyl)thio]acetonitrile in 140 ml of ethanol, stirred ovenight, and neutralized with acetic acid. To this solution of 2-[(4-chlorophenyl)thio]ethanimidic acid, ethyl ester (**4b**) was added a solution of 9.6 g (0.038 mole) of 4-hydrazino-6-[(phenylmethyl)thio]-2,5-pyrimidinediamine (**5**) [5] in 80 ml of dioxane. After the mixture had been stirred 23 hours, the precipitate which had formed was collected and washed with ethanol and with ether to give 2.42 g of fairly pure **6b**, mp 187-190°. The filtrate afforded two additional crude crops totaling 4.9 g which were recrystallized from a mixture of acetonitrile and *N,N*-dimethylformamide (4:1) to afford 3.61 g of analytical **6b** as orange shiny crystals, mp 191-193°, total yield, 6.03 g (37%).

Anal. Calcd. for $C_{19}H_{15}ClN_6S_2$: C, 53.45; H, 3.54; N, 19.69; S, 15.02; Cl, 8.31. Found: C, 53.18; H, 3.48; N, 19.76; S, 15.07; Cl, 8.47.

3-[[(4-Chlorophenyl)thio]methyl]pyrimido[5,4-*e*]-1,2,4-triazine-5,7-diamine (**7b**).

To a solution of 5.9 g (0.014 mole) of 3-[[(4-chlorophenyl)thio]methyl]-5-[(phenylmethyl)thio]pyrimido[5,4-*e*]-1,2,4-triazin-7-amine (**6b**) in 210 ml of *N,N*-dimethylacetamide at 60° was added 150 ml of ethanol saturated with ammonia. The mixture was stirred at 60° for 5 hours, chilled and filtered to collect the yellow precipitate which had accumulated. The precipitate was washed with 2-propanol and ether, and dried at 100° *in vacuo* to afford 3.75 g (87%) of **7b**, mp > 280°.

Anal. Calcd. for $C_{12}H_{10}ClN_7S$: C, 45.07; H, 3.15; N, 30.66; S, 10.02;

Cl, 11.09. Found: C, 44.93; H, 3.40; N, 30.52; S, 9.78; Cl, 11.24.

3-[[(4-Chlorophenyl)sulfinyl]methyl]pyrimido[5,4-*e*]-1,2,4-triazine-5,7-diamine, Compound With *N,N*-Dimethylformamide (1:0.2), Hydrate (1:0.2) (**8**).

A mixture of 1.3 g (0.0041 mole) of 3-[[(4-chlorophenyl)thio]methyl]pyrimido[5,4-*e*]-1,2,4-triazin-5,7-diamine (**7b**), 14 ml of 30% hydrogen peroxide and 97 ml of glacial acetic acid was stirred for 1.25 hours at room temperature and then poured into iced, dilute sodium hydroxide solution. The resulting precipitate was collected, washed with water and dissolved in 150 ml of boiling *N,N*-dimethylformamide. The hot solution was filtered, diluted with 5 ml of water, treated with charcoal, filtered through celite, allowed to cool, chilled and filtered to remove 0.3 g of starting material. The filtrate afforded a yellow solid which was collected and dried under vacuum at 90° to give 0.44 g (31%) of **8**, mp 282-285° dec.

Anal. Calcd. for $C_{12}H_{10}ClN_7OS \cdot 0.2C_3H_7NO \cdot 0.2H_2O$: C, 42.75; H, 3.36; N, 28.49; S, 9.06; Cl, 10.02. Found: C, 42.52; H, 3.45; N, 28.91; S, 8.77; Cl, 10.02.

Due to the limited amount of sample, a water analysis was not obtained, however the nmr spectrum confirmed the presence of approximately 0.2 mole of water plus 0.2 mole of *N,N*-dimethylformamide. The presence of the sulfinyl group was evident in the ir spectrum (1046 cm^{-1}). In addition, oxidation of the sulfur creates asymmetry so that the adjacent methylene group which appears as a singlet at 4.58 ppm in the nmr spectrum of **7b** appears as a doublet of doublets at 4.69 ppm in the spectrum of **8**.

N-[(5,7-Diaminopyrimido[5,4-*e*]-1,2,4-triazine-3-yl)methyl]-*N*-(3,4-dichlorophenyl)formamide (**9**).

A solution of 1.0 g (0.003 mole) of 3-[[(3,4-dichlorophenyl)amino]methyl]pyrimido[5,4-*e*]-1,2,4-triazine-5,7-diamine (**7a**), 0.4 hydrate in 50 ml of 97% formic acid was heated under reflux for 4 hours and evaporated to dryness under vacuum. The residue was triturated with ethanol and recrystallized from *N,N*-dimethylformamide-dilute ammonium hydroxide to afford 0.95 g (90%) of **9**, mp 282-284° dec.

Anal. Calcd. for $C_{13}H_{10}Cl_2N_8O$: C, 42.75; H, 2.76; N, 30.69; Cl, 19.42. Found: C, 42.55; H, 2.96; N, 31.20; Cl, 19.43.

The methylene group appears as a singlet at 5.40 ppm in the nmr spectrum.

3-[[(3,4-Dichlorophenyl)nitrosoamino]methyl]pyrimido[5,4-*e*]-1,2,4-triazine-5,7-diamine (**10**).

To a chilled slurry (3°) of 1.35 g (0.04 mole) of 3-[[(3,4-dichlorophenyl)amino]methyl]pyrimido[5,4-*e*]-1,2,4-triazine-5,7-diamine (**7a**), 0.4 hydrate in 150 ml of 90% aqueous acetic acid was added dropwise a solution of 0.28 g (0.004 mole) of sodium nitrite in 2 ml of water. The mixture was allowed to warm to room temperature, stirred overnight and filtered. The filter cake was washed with water and ethanol and then recrystallized from *N,N*-dimethylformamide containing one drop of concentrated ammonium hydroxide to afford 0.53 g (36%) of **10**, mp > 300°.

Anal. Calcd. for $C_{12}H_9Cl_2N_9O$: C, 39.36; H, 2.48; N, 34.43; Cl, 19.37. Found: C, 39.32; H, 2.82; N, 34.38; Cl, 19.67.

The methylene group appears as a singlet at 5.73 ppm in the nmr spectrum.

Acknowledgement.

Synthetic assistance from Linda Newton (intermediate **1**), Jeanne Hofteizer, and Norman Colbry (intermediate **2**) is gratefully acknowledged. The authors are indebted to Dr. C. L.Heifetz and Dr. M. W. Fisher for the antibacterial studies and to Dr. Joan Shillis and coworkers for the L1210 tissue culture evaluation. We also thank Mr. C. E. Childs and associates for the microanalyses, Dr. J. M. Vandenbelt and coworkers for determination of the spectral data.

REFERENCES AND NOTES

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[2] This investigation was supported by U. S. Army Medical Research and Development Command Contract DADA-17-72-C-2077. This is contribution 1743 to the Army Research Program in Malaria.

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