diamine as a Potential Antimalarial Agent [1]

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Received October 17, 1983

The synthesis of 6-ethyl-5-(4-pyridinyl)-2,4-pyrimidinediamine (2), the pyridine analog of pyrimethamine, is described. Condensation of 4-pyridineacetonitrile (3) with methyl propionate afforded α-(1-oxopropyl)-4-pyridineacetonitrile (4). Treatment with triethyl orthopropionate gave primarily the undesired N-alkylated material. However reaction of the crude mixture with guanidine afforded 2 in low yield. This material was devoid of significant antimalarial activity.

Pyrimethamine (1) acts by selectively inhibiting plasmodial dihydrofolate reductase [2-4], and has been used as a suppressive and prophylactic antimalarial drug [5,6] since 1951. Its efficacy has been compromised recently by the occurrence of more frequent resistant plasmodial strains in the field [7-11]. Thus, an improved agent would be highly desirable.

Many compounds related to pyrimethamine have been prepared [12], however analogs in which the phenyl ring is replaced with a heterocyclic ring have not been reported. Since the replacement of a *para*-chloro group on a phenyl ring by a ring nitrogen sometimes leads to retention of biological activity, and since the presence of an additional basic center in the molecule could confer improved binding properties with the target enzyme, it was of interest to prepare 6-ethyl-5-(4-pyridinyl)-2,4-pyrimidinediamine (2), the pyridine analog of pyrimethamine.

In an attempt to model the synthetic pathway after that utilized for pyrimethamine [13], the synthesis of 2 was initiated with (4-chloromethyl)pyridine (Scheme I). Its reaction with sodium cyanide gave 4-pyridineacetonitrile (3) [14] which was treated with sodium hydride and methyl propionate to provide 4 [15]. The infrared spectrum of 4 shows bands characteristic of a conjugated nitrile (2180 cm^{-1}) [16], and either a conjugated ketone or an olefin (1640 cm^{-1}) [17,18]. Of the three possible tautomers of 4, represented as 4a, 4b, and 4c structures 4a and 4b appear to best fit the data.

Attempted condensation of compound 4 with guanidine to provide the target 2 was unsuccessful. In the hope that the enol ether of compound 4 might facilitate the condensation with guanidine, 4 was alkylated with triethyl orthopropionate to provide intermediate 5. Condensation of the purified intermediate with guanidine failed to provide 2. However, when the alkylation intermediate 5 was generated *in situ* and allowed to react with guanidine, compound 2 was obtained in 7% yield along with 90% recovery of intermediate 5.
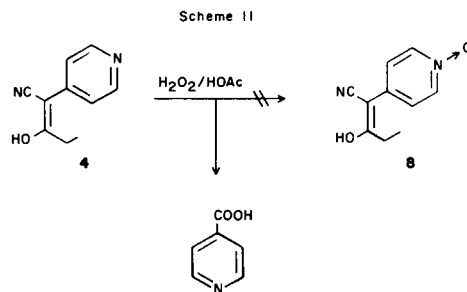

Scheme I
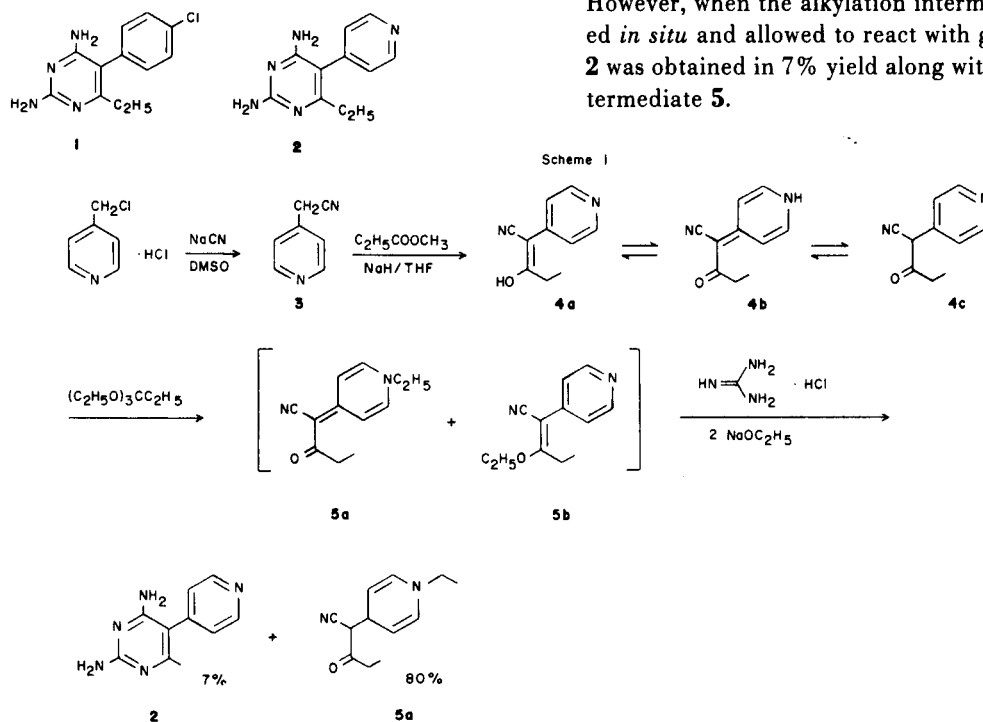
This suggested that the alkylation of **4** with triethyl orthopropionate may have taken place predominantly on the ring nitrogen to give 2-[1-ethyl-4(1*H*)-pyridinylidene]-3-oxapentanenitrile (**5a**) rather than, as expected, on the carbonyl oxygen. The resultant **5a** would then be unable to condense with the guanidine. The crude alkylation mixture however likely contained a small amount of the desired *O*-alkylation product **5b**, thus leading to the formation of a small amount of compound **2** when it was used in the guanidine condensation.

In order to verify that alkylation of **4** occurred predominantly at the ring nitrogen, the infrared and ultraviolet spectra of the single purified product isolated from the reaction mixture was compared with those of the reported compounds **6** and **7** [19] in Table I.


6   7
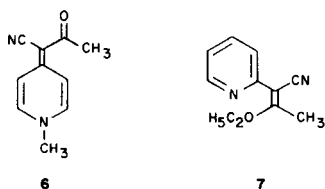
The infrared spectrum of the purified compound **5**, showing bands characteristic for a conjugated nitrile, a conjugated carbonyl, and an olefin, is very similar to that of compound **6**. The intense untraviolet absorption at 373 mμ also suggests the presence of a longer conjugated system in the structure, as in the case of compound **6**. Therefore, these data for compound **5** are best accomodated by the structure of **5a**, the *N*-alkylated product.

Since compound **4** was alkylated predominantly at the ring nitrogen, rather than at the carbonyl oxygen, it was thought that ring alkylation could be prevented by first making the *N*-oxide derivative (*i.e.*, compound **8** in Scheme II). The final product could then be obtained by *O*-alkylation of compound **8** followed by condensation with guanidine and deoxygenation. However, the attempted *N*-oxidation of **4** with hydrogen peroxide in acetic acid led instead to removal of the entire carbon chain and oxidation to pyridine-4-carboxylic acid.
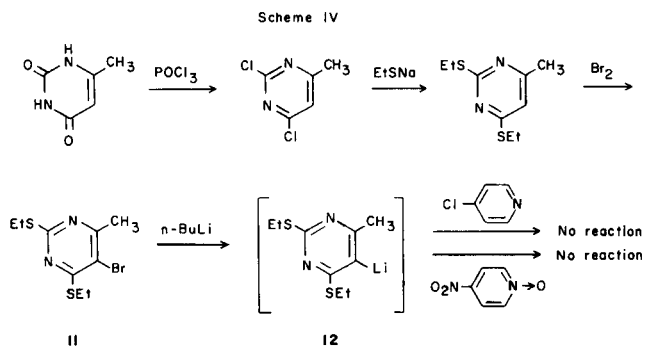
Scheme II

A variety of alternative synthetic approaches to **2** were also explored. Thus either the chloro compound **9** or the corresponding amine **10** could have led to **2** as shown in Scheme III. However chlorination of **4** with thionyl chloride to provide **9** led only to decomposition, and we were unable to form the enamino nitrile **10**, from **3**.
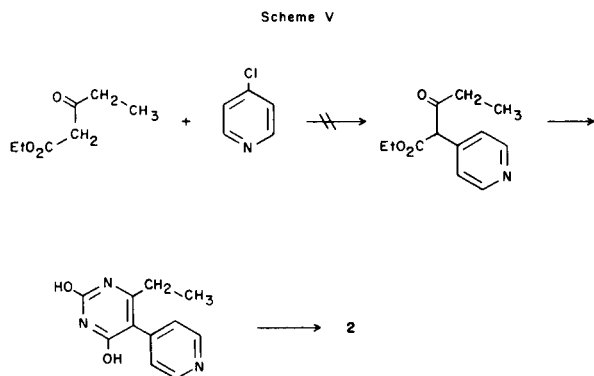
Scheme III
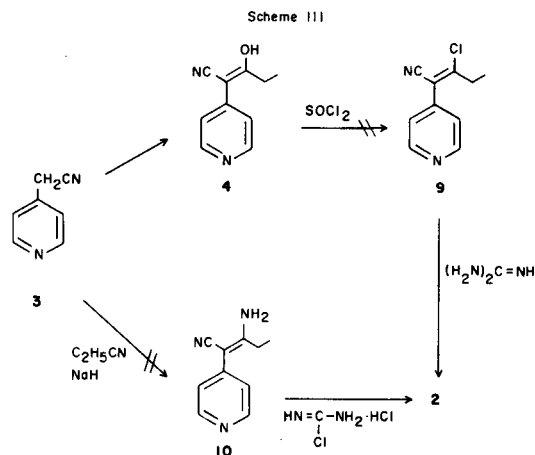
Moreover, compound **11** was prepared from readily available uracil and was used as a model to explore the synthesis of 5-pyridine substituted pyrimidines. However, neither the reaction of 4-chloropyridine nor 4-nitropyridine *N*-oxide with the lithiated intermediate **12** (Scheme IV) could be affected.

Table I

Comparison of Infrared and Ultraviolet Spectra of Compounds
5, 6 and **7**

	5	6	7
IR	2180 cm^{-1}	2220 cm^{-1} (C=C-C≡N)	2220 cm^{-1} (C≡N)
	1650 cm^{-1}	1660 cm^{-1} (C=C-C=O)	1630 cm^{-1} (C=C)
	1600 cm^{-1}	1600 cm^{-1} (C=C)	1585 cm^{-1} (pyridine)
			1560 cm^{-1} (pyridine)
UV	234 mμ (ε 13,307)	235 (15,400)	265 mμ (ε 12,200)
	373 mμ (ε 40,792)	362 (35,000)	287 mμ (ε 14,400)
λ max			362 mμ (ε 1,250)

Scheme IV

In Scheme V the initial condensation with 4-chloropyridine could not be affected.

Scheme V

Thus, this study did effect, albeit in low yield, the synthesis of the desired 6-ethyl-5-(4-pyridinyl)-2,4-pyrimidinediamine, and our studies have indicated the shortcomings of all synthetic schemes attempted thus far.

The target compound **2** was submitted to the Walter Reed Army Institute of Research for evaluation of antimalarial activity [21] in the *Plasmodium berghei* mouse model. Despite its very close similarity to pyrimethamine it was devoid of substantial biological activity.

EXPERIMENTAL

Melting points were determined on a Thomas-Hoover capillary melting point apparatus and were uncorrected. Proton magnetic resonance (^{1}H nmr) spectra were obtained using a Varian Associates EM-390 or Bruker B-NC-12 instrument and ^{13}C nmr Spectra were run on a Bruker WH90 spectrometer. Chemical shifts are recorded in parts per million (δ) relative to tetramethyl silane as internal standard. The ir spectra were determined on a Digilab FTS14 or Nicolet MX1 spectrophotometer. The elemental analyses were performed on a Perkin-Elmer 240. All starting materials were commercially available.

4-Pyridineacetonitrile (**3**).

This was prepared as described by VonWerner Schulze [14]. Sodium cyanide, 80.0 g (1.63 mole), was added to 250 ml of vigorously stirred dimethyl sulfoxide. The suspension was stirred vigorously and cooled in an ice-bath while 100 g (0.61 mole) or (4-chloromethyl)pyridine monohydrochloride was added in portions so that the reaction temperature did not rise above 30°. After the addition was complete, the resulting mixture was allowed to stir at room temperature for another three hours and then poured into 1200 ml of water. The aqueous solution (pH 7) was ex-

tracted with ethyl acetate (800 ml × 2). The organic layer was separated, washed with water (500 ml) and saturated sodium chloride solution, dried over magnesium sulfate and filtered. The dark brown filtrate was passed through 200 g of fluorosil to remove the colored impurities. The filtrate was then concentrated *in vacuo* at 40° to give a light brown oil, which crystallized upon cooling at 0° for 16 hours to give 61.8 g (85%) of the product as a light brown solid, mp 41-43° (lit [14] 40°). The product was used immediately in the next reaction

α-(1-Oxopropyl)-4-pyridineacetonitrile (**4**).

Sodium hydride, 15.8 g (0.33 mole, 50% dispersion in mineral oil), was added portionwise to a cold solution of 36.0 g (0.30 mole) of 4-pyridineacetonitrile (**3**) and 28.9 g (0.33 mole) of methyl propionate in 330 ml of *N,N*-dimethylformamide. After the bubbling stopped, the solution was heated at reflux for 24 hours and cooled. The solution was poured into 1200 ml of ice water and neutralized with 6N aqueous hydrochloric acid to pH 7. The precipitate was collected, washed with 500 ml of water and recrystallized from acetonitrile to give 25.0 g (47%) of the product as a pink solid, mp 195-200°; ir (potassium bromide): 2180 cm^{-1} (C≡N stretching of > C=C-C≡N from structure **4a** or **4b**), 1640 cm^{-1} (C=C stretching of **4a** or C=O stretching of **4b**), 1535 cm^{-1} (pyridine); ^{1}H nmr (DMSO-d$_6$): δ 1.00 (t, 3H, CH$_3$), δ 2.50 (q, 2H, CH$_2$), δ 7.60 ~ 7.90 (broad, 4H, aromatic), δ 12.30 ~ 13.00 (broad, 1H, exchangeable).

Anal. Calcd. for C$_{10}$H$_{10}$N$_2$O: C, 68.94; H, 5.79; N, 16.08. Found: C, 68.73; H, 5.97; N, 15.85.

O-Alkylation *versus* *N*-Alkylation of α-(1-Oxopropyl)-4-pyridineacetonitrile.

A mixture of 8.60 g (0.049 mole) of α-(1-oxopropyl)-4-pyridineacetonitrile (**4**) in 30 ml of triethyl orthopropionate was heated at 80° for three hours, with continuous removal of the low boiling liquid (ethanol and propionic acid). The reaction mixture was concentrated *in vacuo* at 70° to give a black oily residue which was poured into 200 ml of ice water. The solid precipitate was collected and recrystallized from acetonitrile to give 8.04 g (80%) of an off-white solid, mp 195-196°; ^{1}H nmr (deuteriochloroform): δ 1.10 (t, 3H, C-CH$_2$C*H$_3$*), δ 2.60 (q, 2H, C-C*H$_2$*), δ 3.90 (q, 2H, NCH$_2$); δ 7.30 (d, 2H, aromatic), δ 7.30 ~ 9.00 (broad, 2H, aromatic); ^{13}C nmr (deuteriochloroform): 195 ppm (C=O); ir (chloroform): 2180 cm^{-1} (C≡N stretching of C=C-C≡N, 1650 cm^{-1} (C=O stretching of C=C-C=O) and 1600 cm^{-1} (C=C).

The presence of a conjugated nitrile, a conjugated carbonyl and an olefin in the infrared spectrum suggested that the structure of the product is **5a**.

Anal. Calcd. for C$_{12}$H$_{14}$N$_2$O: C, 71.26; H, 6.98; N, 13.85. Found: C, 71.20; H, 6.97; N, 13.86.

6-Ethyl-5-(4-pyridinyl)-2,4-pyrimidinediamine (**2**).

α-(1-Oxopropyl)-4-pyridineacetonitrile, 23.2 g (0.13 mole), was alkylated with 300 ml of triethyl orthopropionate as described above. The reaction mixture was concentrated *in vacuo* at 70° to dryness and the oily residue was used directly in the following reaction without purification. Sodium (6.14 g, 0.26 mole), was dissolved in 500 ml of ethanol, 12.7 g (0.13 mole) of guanidine hydrochloride was added and the resulting mixture was stirred at room temperature for 30 minutes. The black oily residue from above was added and the resulting mixture was heated at reflux for 16 hours. The reaction mixture was filtered hot and the solid was washed first with water and then with ethanol and filtered. Trituration of the solid with 50 ml of boiling *N,N*-dimethylformamide gave 2.24 g (7%) of the product as a dull yellow solid, mp > 330°; ^{1}H nmr (trifluoroacetic acid): 1.15 ~ 1.45 (t, 3H, CH$_3$), δ 2.40 ~ 2.75 (q, 2H, CH$_2$), δ 6.60 ~ 7.80 (broad, 4H, NH$_2$), δ 8.10 ~ 8.25 (d, 2H, C$_2$'-H), δ 8.90 ~ 9.10 (d, 2H, C$_3$'-H).

Anal. Calcd. for C$_{11}$H$_{13}$N$_5$: C, 61.38; H, 6.09; N, 32.54. Found: C, 61.26; H, 6.16; N, 32.49.

The filtrate from the reaction mixture was concentrated *in vacuo* to dryness at 80°. The solid residue was recrystallized from acetonitrile to give 21.0 g (90%) of a fluffy tan solid, mp 195-197°. The ir and nmr spectra of this solid are identical with those of the purified alkylation product

from α-(1-oxopropyl)-4-pyridineacetonitrile.

2,4-Dichloro-6-methylpyrimidine.

A mixture of 12.6 g (0.1 mole) of uracil and 150 ml of phosphorus oxychloride was heated under reflux for three hours. Phosphorus oxychloride was removed *in vacuo*. The gummy residue was poured into 500 ml of ice water and dried *in vacuo* at 40° overnight to give 13.5 g (83%) of the product as a light yellow solid, which was used directly in the next reaction without further purification.

2,4-Bisethylthio-6-methyl-pyrimidine.

This was prepared as described by Sprague and Johnson [20].

Sodium, 2.64 g (0.114 mole) was added piece by piece to a cooled solution of 7.1 g (0.114 mole) of ethylthiol in 50 ml of toluene. The mixture was stirred at ice bath temperature for two hours until sodium disappeared. A solution of 8.5 g (0.052 mole) of 2,4-dichloro-6-methylpyrimidine in 10 ml of toluene was then added. The reaction mixture was heated under reflux for 16 hours and cooled. It was poured slowly into 200 ml of ice water and extracted with ethyl ether (200 ml × 2). The organic layer was washed successively with water (200 ml) and saturated sodium chloride solution (100 ml), dried over magnesium sulfate, and concentrated *in vacuo* to give 9.8 g (88%) of the product as a light yellow oil; ^{1}H nmr (deuteriochloroform): δ 1.2 - 1.45 (m, 6H, 2CH$_3$), δ 2.2 (s, 3H, CH$_3$), δ 2.9-3.3 (m, 4H, 2S CH$_2$), δ 6.5 (S, 1H, C$_5$-H).

2,4-Bisethylthio-5-bromo-6-methylpyrimidine (11).

A solution of 7.3 g (0.046 mole) of bromine in 10 ml of methylene chloride was added dropwise to a cooled solution of 9.8 g (0.046 mole) of 2,4-bisethylthio-6-methyl pyrimidine in 40 ml of methylene chloride at an ice bath temperature. After the addition, the reaction mixture was stirred for another hour. Methylene chloride was removed *in vacuo*. The solid residue was washed with 50 ml of water and recrystallized from acetonitrile to give 12.7 g (94%) of the product as a light yellow solid, mp 43-45°.

Attempted Synthesis of 2,4-Bisethylthio-6-methyl-5-(4-pyridinyl)pyrimidine.

A solution of 3.20 ml (3.60 mole) of a 1.125M solution of *n*-butyl lithium in *n*-hexane was added dropwise to a solution of 1.0 g (3.40 mmole) of 2,4-bisethylthio-5-bromo-6-methylpyrimidine (12) in 40 ml of dry tetrahydrofuran at -78°. The mixture was stirred at that temperature for one hour. A solution of 0.39 g (3.4 mole) of 4-chloropyridine in 10 ml of tetrahydrofuran was added and the mixture was allowed to warm up to room temperature. The reaction mixture was cooled to -60° and a saturated ammonium chloride solution was added. After warming up to room temperature the mixture was poured into 200 ml of ice water. The solid isolated was characterized as starting materal 11.

The same result was observed when 4-chloropyridine was replaced by 4-nitropyridine *N*-oxide in the reaction.

REFERENCES AND NOTES

[1] This is communication 54 of a series on antimalarial agents. For paper **53** see L. M. Werbel, E. F. Elslager and J. Johnson, *J. Med. Chem.*, in press. This investigation was supported by U. S. Army Medical Research and Development Command Contract DAMD-17-79-C9115. This is contribution Number 1689 to the Army Research Program on Malaria.

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[21] The parenteral suppressive antimalarial screening was carried out by Dr. Arba L. Ager, Jr. at the University of Miami and test results were provided through the courtesy of Dr. T. R. Sweeney, Dr. E. A. Steck, and Mr. M. Musallam of the Walter Reed Army Institute of Research for Malaria. For a description of the test method, see T. S. Osdene, P. B. Russell and L. Rowe, *J. Med. Chem.*, **57**, 2252 (1935).