Synthesis of Some 1,3,8-Trisubstituted Pyrimido[4,5-*c*][2,7]-naphthyridin-6-ones as Potential Antitumor Agents

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Condensation of three 2,4-disubstituted 6-aminopyrimidines with methyl 1-benzyl-4-oxo-3-piperidinecarboxylate afforded, in each case, new tricyclic, angular 1,3,8-trisubstituted pyrimido[4,5-*c*][2,7]naphthyridin-6-ones. 2,4,6-Triaminopyrimidine gave the 7,8,9,10-tetrahydrocyclo condensed product **5** as anticipated. However, the use of 2-amino-4-oxo- or 2,4-dioxo-6-aminopyrimidine afforded the dehydrogenated, 9,10-dihydrotricyclic products **11** and **12**. The growth of leukemia L1210 cells in culture were inhibited 50% by the 1,3-diamino analog **5** at $2 \times 10^{-6}M$ and by the 1,3-dioxo analog **12** at $10^{-5}M$.

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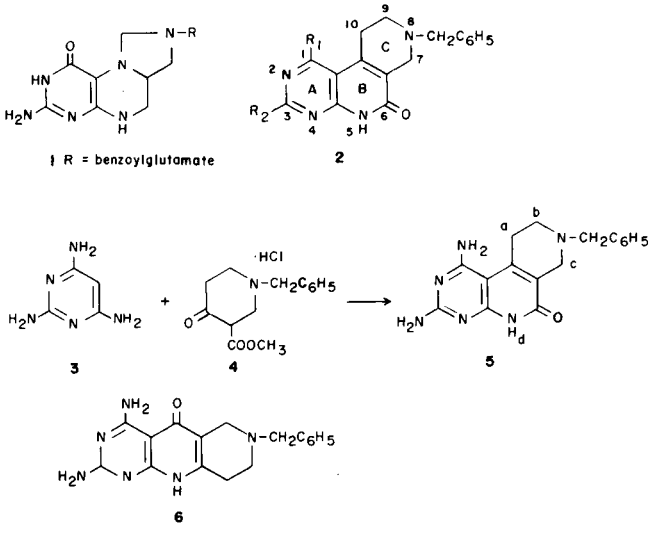
As part of our research effort directed towards the synthesis of 5-deaza analogues and homologues of the folate cofactor, 5,10-methylenetetrahydrofolate **1** [1], we were interested in the synthesis of tricyclic 1,3,8-trisubstituted pyrimido[4,5-*c*][2,7]naphthyridin-6-ones of general structure **2** as potential antitumor agents.

1,3-Diamino-7,8,9,10-tetrahydropyrimido[4,5-*c*]isoquinoline [2,3] and other similar tricyclic systems [3] have been synthesized and their antitumor and antifolate activity reported. However, these systems lack a nitrogen in the C-ring and were inappropriate for our purpose.

Since **2** represents a new heterocyclic ring system there was no literature precedent for its synthesis. However, a one step facile entry into the pyrimido[4,5-*c*][2,7]naphthyridin-6-one ring system could be achieved *via* the cyclocondensation of an appropriately substituted 6-aminopyrimidine with a β-ketoester using a modification of the procedure reported for 5,6-disubstituted pyrido[2,3-*d*]pyrimidin-6-ones [2].

Condensation of 2,4,6-triaminopyrimidine (**3**) with methyl 1-benzyl-4-oxo-3-piperidinecarboxylate (**4**) in glacial acetic acid afforded, following basification, 1,3-diamino-8-benzyl-7,8,9,10-tetrahydropyrimido[4,5-*c*][2,7]naphthyridin-6(5*H*,8*H*)-one (**5**). This cyclocondensation reaction can occur with the 5-position of the pyrimidine **3** being attached to the ketone carbonyl of **4** and the 6-amino (or 4-amino) moiety being attached to the ester carbonyl of **4** to give the angular isomer **5**. However, an alternative mode of condensation is also possible, which would afford the linear isomer **6**.

Literature evidence suggests that under acidic conditions substituted 6-aminopyrimidines and 5-aminopyrazoles undergo similar reactions with β-dicarbonyl compounds such that the more reactive carbonyl carbon (the ketone in **4**) is attached to the carbon β to the amino moiety in the cyclocondensed product [2,4-7], thus favoring structure **5**.



Additional support for the angular structure **5** was provided from ^{13}C nmr spectral data. Ratajezyk and Swett [5] and Winn [6] have reported a significant difference in the chemical shifts of the carbonyl carbons in isomeric fused pyridones. Fused α-pyridones have the largest chemical shift corresponding to the carbonyl carbon in the range of δ 162.3-164.5 while in fused γ-pyridones the carbonyl carbon signal occurs in the range of δ 178.5-177.9. Compound **5** (a fused α-pyridone) had the largest chemical shift at δ 166.03 assigned to the carbonyl carbon which strongly favors the angular structure depicted as **5**.

The ^1H nmr spectra of **5** was also consistent with its structure. In particular, the chemical shifts and nature of the signals of protons *a*, *b*, *c* and *d* of **5** should be noted. In deuteriotrifluoroacetic acid protons *a* occur as a multiplet centered at δ 2.80 (Figure I A), protons *b* and *c* occur as a multiplet at δ 3.77 (Figure I A) and proton *d* is exchanged. In DMSO-d_6 the lactam proton *d* occurs at δ 8.61 and exchanges on addition of deuterium oxide.

Having established the mode of cyclocondensation and

the structure of the product as the angular isomer **5** we decided to synthesize the 3-amino-1-oxo and 2,4-dioxo derivatives of **5** in view of their potential activity as antitumor agents.

On condensation of **7** (R = NH_2) and **8** (R = OH) with **4** we anticipated **9** and **10** respectively. However, the ^1H nmr spectra of purported **9** and **10** were inconsistent with the assigned structures. An unexpected nonexchangeable proton appeared at δ 9.40, as a sharp singlet, for the cyclocondensation product from **7** and at δ 9.43 for the product from **8**. Since these protons in each case were nonexchangeable in deuteriotrifluoroacetic acid they could not be assigned to the lactam proton *d* in **9** and **10**. This lactam proton *d* was absent in both spectra.

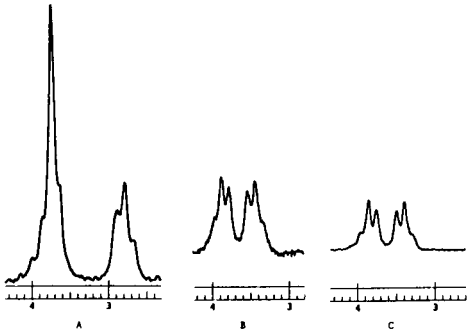
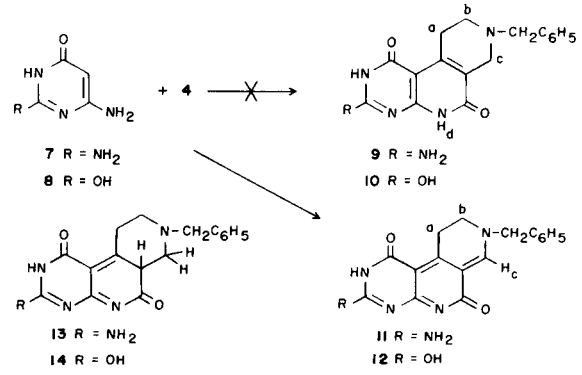


Figure 1. Methylene region of the ^1H NMR Spectra of **5** (A), **11** (B) and **12** (C).

Further, the methylene region of the ^1H nmr spectra for **9** and **10** corresponding to protons *a*, *b* and *c* integrated for only four protons (compared to six protons for **5**), and formed centrosymmetric patterns (Figure I B for R = NH_2 and Figure I C for R = OH) characteristic of AA'BB' systems, and hence must be assigned to vicinal protons *a* and *b*. Protons *c* were therefore the two missing protons in the methylene region compared to the ^1H nmr spectra of **5**. The benzyl methylene protons were at δ 4.93 and 4.94 for **9** and **10** respectively as expected.

Compared to the ^1H nmr spectrum of **5** the purported spectra of **9** and **10** differed in three essential features. The absence of the lactam proton *d*, the absence of a pair of methylene protons *c* and the presence of an olefinic proton. This suggested that a total loss of two hydrogens had occurred for each of the products from **7** and **8** compared to **5** and that the products could not be **9** and **10** as designated.

Based on the ^1H nmr spectra described above and literature precedent of unusual dehydrogenation occurring during similar types of cyclocondensations [6] we assigned structures **11** and **12** to the products from the reaction of **4** with **7** and **8** respectively. These structures could arise from air oxidation of intermediates **13** and **14**. The downfield nonexchangeable, olefinic proton is assigned to



H_c in **11** (δ 9.40) and **12** (δ 9.43). The AA'BB' portion of the spectrum of **11** and **12** results from vicinal methylene protons *a* and *b*.

It is possible that the 1-oxo moiety allows for additional overlap involving lone pair of electrons on the N^8-nitrogen thus stabilizing structures **11** and **12** in preference to **9** and **10** respectively. Such an additional overlap would not be present in **5**, and could, in part, account for the difference in the structure of the cyclocondensation product.

The growth of leukemia L-1210 cells in culture [8] was inhibited 50% by **5** at 2×10^{-6} *M* and by **12** at 10^{-5} *M*. Compound **11** was inactive at these concentrations. In L-1210 cells *in vitro* none of the compounds showed any inhibition in the tritium release assay [9] at 10^{-4} *M*, 10^{-5}*M* and 10^{-5} *M* for **5**, **11** and **12** respectively. Using permeabilized L-1210 cells [10] only **5** inhibited dihydrofolate reductase by 30% at 10^{-4} *M* and no inhibition of thymidylate synthetase by any of the compounds could be detected.

Modified analogues of the compounds described, in particular of **5**, are currently being explored in order to gain information with regard to the mechanism of action and to develop better inhibitors of various tumor cell systems.

EXPERIMENTAL

Melting points were determined in capillary tubes on a Fisher-Johns melting point apparatus and are uncorrected. Infrared spectra (ir) were recorded with a Perkin-Elmer Model 337 in potassium bromide discs. Nuclear magnetic resonance spectra for proton (^1H-nmr) were run on a Varian EM-360 and for carbon-13 (^{13}C-nmr) on a Varian FT-80 with internal standard TMS; s = singlet, d = doublet and m = multiplet. thin-layer chromatography (tlc) was performed on silica-gel plates with fluorescent indicator and were visualized with light at 254 nm. The elemental analysis were performed by Galbraith Laboratories in Knoxville, Tennessee.

1,3-Diamino-8-benzyl-7,8,9,10-tetrahydropyrimido[4,5-*c*][2,7]naphthyridin-6(5*H*,8*H*)-one (**5**).

A mixture of 2.5 g (0.02 mole) of 2,4,6-triaminopyrimidine, 5.6 g (0.02 mole) of methyl 1-benzyl-4-oxo-3-piperidinecarboxylate hydrochloride and 125 ml of glacial acetic acid was stirred and heated to reflux. After 45 minutes a pale yellow solid separated until a thick slurry was obtained. Reflux was continued for 2 hours after which 2 ml of solvent was distilled at atmospheric pressure and reflux continued for an additional 12 hours. After cooling and removal of glacial acetic acid under vacuum the resulting acetate salt was recrystallized from glacial acetic acid, suspended in

water and made basic with 5 *N* sodium hydroxide. The solid was filtered, washed with water until neutral and dried (phosphorus pentoxide) under vacuum to give 4.6 g (70%) of **5** as an orange powder. The compound was homogeneous by tlc on silica gel with ethyl acetate-methanol (1:1 v/v), ethanol-water-0.1 *N* hydrochloric acid (4:1:1 v/v) and ethanol-water-pyridine (4:1:1 v/v). An analytical sample was prepared by recrystallization from dimethylformamide; ir (potassium bromide): 3430, 3380, 3350, 3255 (NH_2), 3175 (NH), 1660 cm^{-1} (C=O); ^1H nmr (DMSO-d_6): δ 3.0-3.33 (m, 4H, CH_2NCH_2), 4.53 (s, 2H, $CH_2C_6H_5$), 5.50 (broad s, 2H, NH_2), 5.97 (broad s, 2H, NH_2), 7.26 (s, 5H, C_6H_5), 8.61 (broad s, 1H, NHCO); ^1H nmr (deuteriotrifluoroacetic acid): δ 2.6-3.2 (m, 2H, CH_2), these protons overlap with the DMSO signal and cannot be distinguished from it in the spectra using DMSO-d_6 as solvent.

Anal. Calcd. for $C_{17}H_{18}N_6O\cdot0.5H_2O$: C, 61.62; H, 5.78; N, 25.36. Found: C, 61.29; H, 5.77; N, 25.31.

3-Amino-8-benzyl-1-oxo-9,10-dihydro-2*H*-pyrimido[4,5-*c*][2,7]naphthyridin-6(8*H*)-one (**11**).

A mixture of 6.4 g (0.05 mole) of 2,4-diamino-6-hydroxypyrimidine, 14.9 g (0105 mole) of methyl 1-benzyl-4-oxo-3-piperidinecarboxylate hydrochloride and 125 ml of glacial acetic acid was stirred and heated to reflux for 18 hours. Following this 70 ml of solvent was distilled from the reaction mixture. Reflux was continued for an additional 6 hours. The reaction mixture was filtered hot and washed with hot glacial acetic acid. The air dried acetate salt was slurried in 100 ml of water and treated with 5 *N* sodium hydroxide to a *p*H of 12. Filtration followed by washing of the residue with water until neutral and then with diethyl ether afforded the free base as a tan solid. Drying (phosphorus pentoxide) under vacuum gave 6.4 g (40%) of **11** as a tan powder. The compound was homogeneous by tlc on silica-gel with ethanol-water (4:1 v/v); ir (potassium bromide): 3320, 3240 (NH_2), 3110 (NH), 1680 cm^{-1} (C=O); ^1H nmr (deuteriotrifluoroacetic acid): δ 3.43 (t, 2H, $-CH_2-$), 3.85 (t, 2H, CH_2-N), 4.93 (s, 2H, $CH_2C_6H_5$), 7.37 (s, 5H, C_6H_5), 9.40 (s, 1H, NCH=).

Anal. Calcd. for $C_{17}H_{15}N_5O_2\cdot0.3H_2O$: C, 62.49; H, 4.81; N, 21.43. Found: C, 62.43; H, 4.96; N, 21.23.

8-Benzyl-1,3-dioxo-9,10-dihydro-2*H*,4*H*-pyrimido[4,5-*c*][2,7]naphthyridin-6(8*H*)-one (**12**).

A mixture of 6.4 g (0.05 mole) of 4-amino-2,6-dihydroxypyrimidine, 14.9 g (0.05 mole) of methyl 1-benzyl-4-oxo-3-piperidinecarboxylate hydrochloride and 50 ml of glacial acetic acid were stirred and heated to reflux for 18 hours. Following this 25 ml of solvent was distilled, at atmospheric pressure, from the reaction mixture. Reflux was continued for an additional 51 hours. The reaction mixture was filtered hot, washed with hot glacial acetic acid to afford a white solid which was suspended in water and made basic with 0.2 *N* sodium hydroxide until a *p*H of 10, filtration and washing with water until neutral and then with diethyl ether followed by drying (phosphorus pentoxide) under vacuum for 48 hours afforded 4.9 g (30%) of **12** as a white powder. The compound was homogeneous by tlc on silica-gel with ethanol-water (4:1 v/v); ir (potassium bromide): 3240 (NH), 1720, 1765, 1640 (C=O) cm^{-1}; ^1H nmr (deuteriotrifluoroacetic acid): δ 3.40 (t, 2H, CH_2), 3.85 (t, 2H, CH_2-N), 4.94 (s, 2H, $CH_2C_6H_5$), 7.37 (s, 5H, C_6H_5), 9.43 (s, 1H, NCH=).

Anal. Calcd. for $C_{17}H_{14}N_4O_3\cdot0.25H_2O$: C, 62.48; H, 4.47; N, 17.14. Found: C, 62.77; H, 4.86; N, 17.11.

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