Synthesis of Some 2,4-Disubstituted Tetrahydropyrimido[4,5-*b*]quinolines as Potential Antitumor Agents [1]

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Cyclocondensations of two 2,4-disubstituted 6-aminopyrimidines with 2-chloro-1-cyclohexenecarboxaldehyde afforded in each case a new regiospecific synthesis of tricyclic, linear disubstituted 6,7,8,9-tetrahydropyrimido[4,5-*b*]quinolines **2** and **3** in excellent yields. The linear structures and hence the regiospecificity of the synthesis were established using ^{1}H nmr and ^{13}C nmr. The growth of leukemia L1210 cells in culture was inhibited 50% by **2** at 30 × $10^{-6}M$ and 48% by **3** at 100 × $10^{-6}M$.

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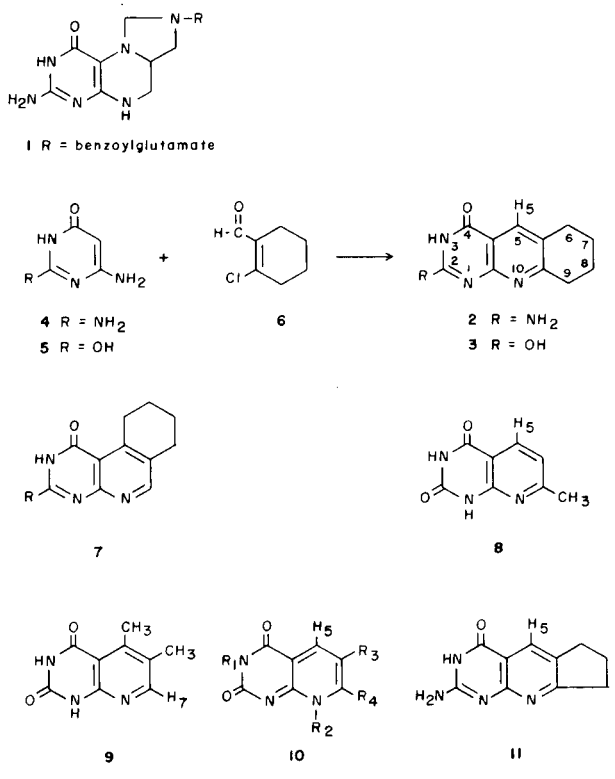
As part of our continuing interest in the synthesis of 5-deaza analogs and homologs of the folate cofactor, 5,10-methylenetetrahydrofolate **1** [2], we have synthesized two linear, 2,4-disubstituted-6,7,8,9-tetrahydropyrimido[4,5-*b*]-quinolines as potential antitumor agents. The recent report of the significant inhibition of dihydrofolate reductase by 5-deazafolic acid [3] lends further support for the synthesis of the 2-amino-4-oxo compound **2** as a nonclassical analog of 5-deazafolic acid. In addition the 2,4-dioxo compound **3** was synthesized as an annulated analog of 2,4-dioxopyrido[2,3-*d*]pyrimidine which has demonstrated antitumor activity [4].

The most direct and simple synthetic entry into the pyrimido[4,5-*b*]quinoline ring system appeared to be the cyclocondensation of an appropriately substituted 6-aminopyrimidine with a cyclohexane containing a 1,3-bis electrophile. Such a cyclocondensation reaction avoids the tedious multistep synthesis of tricylcic compounds which builds two of the three rings in a stepwise manner. However, the one step cyclocondensation reactions can afford linear and/or angular products. The structure of the product is dictated by the direction of the ring closure [2,5].

The synthesis of compound **3** has been reported in 1958 by Robins and Hitchings (28% yield) [6]. These workers employed the cyclocondensation of 2,4-dioxo-6-aminopyrimidine **5** with a ketoaldehyde, 2-formylcyclohexanone, as the biselectrophile. The direction of ring closure and consequently the linear structure of the product was established by an independent synthesis. In the absence of spectral data, the linear structure of the product has been considered by other workers to be arbitrarily assigned [7]. Similar controversies have existed with regard to the direction of ring closure. There are two instances in the recent literature [8a,b,9a,b] where the initial structural assignments of similar cyclocondensation products were found to be erroneous, upon reinvestigation. Since compound **2** has not been synthesized previously and the reported synthesis of **3** gave poor yields and was reported without spectral data we decided to investigate another 1,3-biselectrophile, the chlorovinyl aldehyde **6**, for the synthesis of our target compounds.



2-Chloro-1-cyclohexenecarboxaldehyde (**6**) which served

as the biselectrophile in our synthetic sequence was synthesized by a one step chloroformylation of cyclohexanone according to a literature procedure [10]. Cyclocondensation of 2,6-diamino-4-hydroxypyrimidine (**4**) with **6** in glacial acetic acid regiospecifically afforded only one of the two possible isomeric products, 2-amino-4-oxo-6,7,8,9-tetrahydro-3*H*-pyrimido[4,5-*b*]quinoline (**2**), in excellent yield (90%). Similarly the cyclocondensation of 6-amino-2,4-dihydroxypyrimidine (**5**) with **6** also gave regiospecifically the linear isomer, 2,4-dioxo-6,7,8,9-tetrahydro-1*H*,3*H*-pyrimido[4,5-*b*]quinoline (**3**), in excellent yield (92%). In each case the reaction gave a single product as determined on tlc. The use of trifluoroacetic acid or dimethylsulfoxide in place of acetic acid as solvent afforded a 65% and 52% yield of **3** respectively.

Having synthesized compounds **2** and **3** it became necesary to establish the linear structure of the products. Literature evidence suggests that under acidic conditions aniline [11] and 6-alkylaminouracils [12] with substituted chlorovinylaldehydes undergo similar cyclocondensation reactions such that the amino moiety is attached to the β-carbon of the chlorovinylaldehyde in the cyclocondensed product. This mode of cyclocondensation supports the assigned linear structures **2** and **3**.

Further support for the linear structures was provided from ^{1}H nmr spectra in particular with respect to the chemical shift of the H_5 proton, and from ^{13}C nmr spectral data. Wood *et al.*, [8b] have synthesized 2,4-dioxo-7-methyl-1*H*,3*H*-pyrido[2,3-*d*]pyrimidine (**8**) and reported the H_5 proton at δ 9.05. The 5,6-dimethyl isomer **9** was reported to have the H_7 proton at δ 8.53 [8b] indicating a δ 0.52 difference between the positions of H_5 and H_7 protons of the two compounds in deuteriotrifluoroacetic acid. Both Wood *et al.*, [8b] and Yoneda *et al.*, [12] have reported several 8-substituted pyrido[2,3-*d*]pyrimidines of general structure **10** where the H_5 protons always occurred close to δ 9.00 in deuteriotrifluoroacetic acid [13]. In addition, in a reinvestigation of a report by Stark and Breitmaier [14], Taylor and Fletcher [15] have recently published a multistep synthesis of the 2-amino-4-oxo linear compound **11** in which the H_5 proton was reported to occur at δ 8.9 in deuteriotrifluoroacetic acid. These reports indicate that both the 2-amino-4-oxo compounds and the 2,4-dioxo compounds have their H_5 proton positions close to δ 9.00 in deuteriotrifluoroacetic acid. Compounds **2** and **3** both had their H_5 proton signals at δ 8.93. This further supports the linear structures of compounds **2** and **3**. All other aspects of the ^{1}H nmr were as expected for **2** and **3**. The ^{13}C nmr spectra of **2** and **3** provided additional proof for the linear structures. It has been shown that in pyridines, quinolines [16] and in pyrido[2,3-*d*]pyrimidines [17] the methine carbon γ to the nitrogen, of the pyridine ring, has a one bond coupling constant $^1J_{C\gamma\text{-}H}$ of 157-164 Hz which is approximately 15 Hz less than the one bond coupling constant $^1J_{C\alpha\text{-}H}$ of the carbon α to the nitrogen which is about 177-180 Hz. The proton coupled ^{13}C nmr spectrum of **2** (in deuteriotrifluoroacetic acid-dimethylsulfoxide-d_6, 1:4, v/v), had a doublet for C_5 centered at δ 141.90 with a one bond coupling ($^1J_{C5\text{-}H}$) of 166.0 Hz. Similarly the proton coupled ^{13}C nmr spectrum of **3** in dimethylsulfoxide-d_6 had a doublet for C_5 centered at δ 136.08 with a one bond coupling ($^1J_{C5\text{-}H}$) of 163.67 Hz. The magnitude of both methine coupling constants as well as the chemical shifts support a γ methine carbon (C_5) rather than an α methine carbon (C_7) and consequently, the linear structures **2** and **3** as designated.

In view of the fact that previous literature reports attest to the erroneous structural assignments of similar cyclocondensation reactions [8a,b,9a,b,14], we sought to provide unequivocal proof of our structural assignment by undertaking an x-ray crystal structure determination of compound **3**. This crystal study [18] does indeed show that the compound has the designated linear structure **3**.

We have also repeated the synthesis and thus confirmed that Robins' and Hitchings' 1958 synthesis [6] does afford a compound, *al beit* in low yield, identical in all respects with compound **3**.

To our knowledge this is the first intentional reported synthesis of **2**. In a recent study [19] we have reported the synthesis of the angular isomers of **2** and **3** and thus have been aware, for some time, that the structures of the purported angular compounds reported by Stark and Breitmaier [14] were incorrect and that these workers had inadvertently synthesized the linear analogs **2** and **3** instead of the reported angular isomers. This has been recently confirmed [15].

Based on the above discussion we conclude that the cyclocondensation of **6** with 6-aminopyrimidines **4** and **5** occurs regiospecifically in glacial acetic acid and affords the linear isomers **2** and **3** respectively in excellent yield. A mechanism of the cyclocondensation probably follows that suggested by Gagan and Lloyd [11] for aromatic amines and chlorovinylaldehydes.

The growth of leukemia L1210 cells in culture [20] was inhibited 50% by **2** at $30 \times 10^{-6}M$ and inhibited 48% by **3** at $100 \times 10^{-6}M$. Modifications of **2** and **3** containing substitutions in the C-ring are currently being explored as potential antitumor agents. In addition the corresponding 2,4-diamino analogs are also being pursued.

EXPERIMENTAL

Melting points were determined on a Fisher-Johns melting point apparatus and are uncorrected. Infrared spectra (ir) were recorded with a Perkin-Elmer Model 337 in Nujol mulls. Nuclear magnetic resonance spectra for proton (^{1}H nmr) were run on a Varian EM-360 and for carbon-13 (^{13}C nmr) on a Brucker WH-300 at 75.46 MHz, 90° pulse, 14 μsec. The data was accumulated by 16 K size with 0.5 sec delay time and

70° tip angle, with internal standard TMS; s = singlet, d = doublet and m = multiplet. Thin layer chromatography (tlc) was performed on cellulose plates with fluorescent indicator and were visualized with light at 254 nm. The elemental analysis were performed by Atlantic Microlabs, Inc., Atlanta, Georgia.

2-Amino-4-oxo-6,7,8,9-tetrahydro-3*H*-pyrimido[4,5-*b*]quinoline (**2**).

To a stirred solution of 3.2 g (0.025 mole) of 2,6-diamino-4-hydroxypyrimidine in 150 ml of glacial acetic acid at 100° was added 3.7 g (0.025 mole) of 2-chloro-1-cyclohexenecarboxaldehyde **6** [9] dropwise over 45 minutes and the reaction mixture heated to reflux for 14 hours. The mixture was then poured into water (100 ml), cooled to room temperature and filtered. The filtrate was brought to *p*H 7 with ammonium hydroxide which allowed a gum to separate. Filtration, followed by washing the residue with water and air drying afforded a solid which was suspended in cold water (150 ml), chilled in an ice bath to 5° and dissolved by the addition of sodium hydroxide pellets. Filtration followed by neutralization of the filtrate to *p*H 7 with 10% hydrochloric acid afforded a precipitate which was filtered, washed with water (50 ml) until neutral and dried (phosphorus pentoxide) under vacuum to give 5.2 g (91%) of **2**. The product was homogeneous by tlc on cellulose with *n*-butanol-acetic acid-water (3:1:3 v/v). An analytical sample was prepared by recrystallization from ethanol-water-hydrochloric acid (17:2:1 v/v); ir (Nujol): 3400-3100, 1725, 1670, 1600 cm^{-1}; ^1H nmr (deuteriotrifluoroacetic acid): δ 2.13 (broad s, 4H, 7 and 8-CH_2), 3.26 (m, 4H, 6 and 9-CH_2), 8.93 (s, 1H, H_5).

Anal. Calcd. for $C_{11}H_{12}N_4O \cdot 0.7HCl \cdot 0.2H_2O$: C, 53.85; H, 5.38; N, 22.83; Cl, 10.11. Found: C, 54.08; H, 5.40; N, 22.64; Cl, 10.17.

2,4-Dioxo-6,7,8,9-tetrahydro-1*H*,3*H*-pyrimido[4,5-*b*]quinoline (**3**). Method A.

To a solution of 6.3 g (0.05 mole) of 4-amino-2,6-dihydroxypyrimidine in 180 ml of glacial acetic acid at 100° was added 7.3 g (0.05 mole) of 2-chloro-1-cyclohexenecarboxaldehyde (**6**) dropwise with stirring over 45 minutes and the reaction mixture heated to reflux for 12 hours. After 15 minutes of reflux the product began to separate as a solid. The reaction was cooled to 25°, filtered and the white residue washed with acetic acid-water (3:2 v/v), and then with water to afford 10.01 g (92%) of **3** as a white solid. The product was homogeneous by tlc on cellulose with butanol-acetic acid-water (3:1:3 v/v). An analytical sample was prepared by recrystallization from ethanol-water-dimethylsulfoxide (1:1:8 v/v) containing 3 drops of glacial acetic acid; ir (Nujol): 3220 (NH), 1725, 1665, 1640 (C=O) cm^{-1}; ^1H nmr (deuteriotrifluoroacetic acid): δ 2.13 (broad s, 4H, 7 and 8-CH_2), 3.18 (m, 4H, 6 and 9-CH_2), 8.93 (s, 1H, H_5).

Anal. Calcd. for $C_{11}H_{11}N_3O_2$: C, 60.82; H, 5.10; N, 19.34. Found: C, 60.81; H, 5.10; N, 19.30.

Method B.

To a solution of 6.3 g (0.05 mole) of 4-amino-2,6-dihydropyrimidine in 100 ml of trifluoroacetic acid kept at 0-5° was added 7.3 g (0.05 mole) of 2-chloro-1-cyclohexenecarboxaldehyde **6** [9] dropwise with stirring over 1 hour. Immediate white precipitation of the product was observed. The stirring was continued for 18 hours at room temperature and the mixture was then poured into 200 ml of water and basified with concentrated ammonium hydroxide. Filtration afforded a white solid which was dissolved in 6*N* sodium hydroxide with the temperature maintained below 15°. Neutralization to a *p*H of 6 was accomplished by the dropwise addition of 30% acetic acid (v/v). This afforded a white solid which was filtered, washed with water and recrystallized as in Method A to afford 7.06 g (65%) of a white solid. This compound was identical in every respect (tlc, ir, ^1H nmr) with **3** prepared by Method A. It was also identical with the condensation product of 6-amino-2,4-dihydroxypyrimidine with the sodium salt of formylcyclohexanone, prepared as described by Robins and Hitchings [6].

Method C.

To a solution of 6.3 g (0.05 mole) of 4-amino-2,6-dihydroxypyrimidine in 100 ml of dimethylsulfoxide at 100° was added 7.3 g (0.05 mole) of **6** dropwise over a 15 minute period. The mixture was heated to reflux for 12 hours and then poured into 200 ml of water. The white solid was collected, washed with water and recrystallized as in Method A to afford 5.65 g (52%) of a product identical with **3** (tlc, ir, ^1H nmr).

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