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New compounds via Mannich reaction of cytosine, paraformaldehyde and cyclic secondary amines

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Abstract—The Mannich reaction of cytosine, paraformaldehyde and cyclic secondary amines in the presence of acetic acid gives 5-(4′-morpholinyl)methylcytosine, 5-(1′-piperidinyl)methylcytosine, 5-(1′-pyrrolidinyl)methylcytosine, 5-(4′-methyl-1′-piperidinyl)-methylcytosine, 5-(3′-methyl-1′-piperidinyl)methylcytosine and 5-(2′-methyl-1′-piperidinyl)methylcytosine. These products are quite different from those obtained via cytosine aminomethylation previously described in the literature.

Due to their antiviral and anticancer properties, 5-substituted pyrimidine nucleosides, including 5-substituted derivatives of cytosine have been the subject of increasing interest for many years.[1,2] On the other hand, there is continuing interest in the synthesis of nucleoside analogues in which the carbon in the heterocyclic base is linked to the nitrogen of the secondary cyclic amines.[3] In view of the above, we prepared a series of new 5-substituted derivatives of cytosine, which may have anticancer and/or antivirus activity.

This letter reports the synthesis of 5-(4′-morpholinyl)methylcytosine **1**, 5-(1′-piperidinyl)methylcytosine **2**, 5-(1′-pyrrolidinyl)methylcytosine **3**, 5-(4′-methyl-1′-piperidinyl)methylcytosine **4**, 5-(3′-methyl-1′-piperidinyl)methylcytosine **5** and 5-(2′-methyl-1′-piperidinyl)-methylcytosine **6**, in which the cyclic amines were introduced through a methylene bridge at position-5 of cytosine via a Mannich reaction.

Aminomethylated derivatives of cytosine have been synthesized and isolated earlier.[4] The Mannich reaction of cytosine using 37% formaldehyde and morpholine or diethylamine yielded bis-1,4 adducts. The reaction in which all the substrates were stirred at room temperature using THF as the solvent resulted in bis-N^4,1-(1′-piperidinyl)methylcytosine and bis-N^4,1-(diethylamino)cytosine. These compounds are rather unstable and in aqueous solutions decompose readily to the starting components.

However, it is known that aminomethylation of uracil gives 5-substituted products.[5,6] Also 5-(4′-morpholinyl)methyl-2-thiouracil was synthesized via the Mannich reaction of 2-thiouracil, paraformaldehyde and morpholine using ethanol as the solvent.[7] The above method with some modifications was adapted to prepare compounds **1**–**6** (Fig. 1).[8]

Modification involved the use of glacial acetic acid in combination with other reagents. The absence of acetic acid in the reaction mixture resulted in only a small amount of the desired product, even if the reaction was refluxed over a long period of time (20 h). The use of other acids (HCl, H_2SO_4) gave unsatisfactory results.

Addition of acetic acid, generally improved the solubility of cytosine in ethanol, which had a considerable influence on the kinetics of the reaction. Secondly, acetic acid can facilitate elimination of water from the intermediate formaldehyde-amine adduct. Thirdly, protonation of the pyrimidine base under acidic conditions was expected to make the pyrimidine ring more susceptible towards the attack of the C-5 atom.[9]

The mechanism of this well known reaction (which can occur in acidic solution through neutral to basic solutions) involves electrophilic attack of the intermediate on position-5 of the amino-oxo tautomer of cytosine. This tautomer is the preferred one in the condensed

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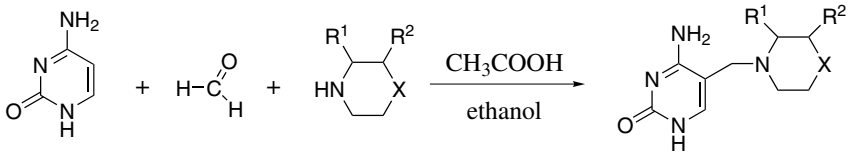

1. R^1 = H, R^2 = H, X = O **2.** R^1 = H, R^2 = H, X = CH_2
3. R^1 = H, R^2 = H, X-null **4.** R^1 =H, R^2 = H, X = $CHCH_3$
5. R^1 = H, R^2 = CH_3, X = CH_2 **6.** R^1 = CH_3, R^2 = H, X = CH_2

Figure 1.

phase, in aqueous solution and even in acidic solution (pH $\leqslant$6), in which the preferred protonation site of cytosine is N3.[10–13]

All reactions were monitored by TLC ($CHCl_3$–CH_3OH 5:1/SiO_2) and also by electrospray ionisation mass spectrometry ESI MS.

To optimize the reaction conditions, ethanol and methanol were used as solvents, with the same stoichiometry of substrates and reaction time, however, when the reaction was conducted in methanol a mixture of products and a small amount of unreacted cytosine were isolated. In all the reactions two equivalents of secondary amine and paraformaldehyde were used and four equivalents of acetic with respect to cytosine. An increase in the equivalents of secondary amine and paraformaldehyde resulted in a decrease of isolated products. It is worth noting that after completion of the reaction and removal of the solvent, the unreacted acetic acid should be carefully evaporated.

Finally, the resulting gummy oil was shaken with dry benzene until a precipitated solid appeared. Dry benzene was a unique solvent for this precipitation (for **1**–**5**) because no other solvent (diethyl ether, hexane, chloroform) worked nearly as well, if at all. After cooling in a refrigerator for a few days while the precipitation was in progress, the crude product was filtered to give pure **1**–**6**. The collected solids did not appear as salts; hence addition of a base was not needed.

The structures of compounds **1**–**6** were determined by comparison of their spectral data with those of 5-methylcytosine[14,15] and cyclic amines.[16–20]

Data from IR, ^{1}H NMR, ^{13}C NMR, ESI MS spectra as well as elemental analyses confirmed the identity of products **1**–**6**.

The ^{1}H NMR spectra (in DMSO-d_6 for **1**, **3**, **4** and in TFA-*d* **1**–**6**) for all the compounds showed a characteristic singlet in the range 7.22–7.36 ppm (DMSO-d_6) or in the range 8.44–8.46 ppm (TFA-*d*) assigned to the proton at C-6 of the cytosine moiety. The ^{1}H NMR spectra in DMSO-d_6 also exhibited two broadened signals at 7.10 and 10.41 ppm, assigned to the protons 4N–H_2 and 1N–H, respectively.

The IR spectra of all compounds (KBr disks) revealed two strong bands in the region 3430–3200 cm^{-1}, which were assigned to ν_{as} (NH_2) and ν_s (NH_2). In the 1600–1700 cm^{-1} range, intense amino group scissoring vibrations α(NH_2) were observed adjacent to the carbonyl bond stretching vibrations ν(C=O).

The ^{13}C NMR spectra were recorded in TFA-*d* solutions, as the solubility of **1**–**6** in DMSO-d_6 and in other solvents was insufficient.[8]

In conclusion it has been shown that reactions of cytosine, paraformaldehyde and cyclic secondary amines in the presence of acetic acid yielded 5-substituted derivatives of cytosine.

This reaction indicates a new route for obtaining similar compounds and calls for further developments. All the obtained compounds are being tested for biological activity.

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8. General procedure for the syntheses of **1**–**6**: Cytosine (9 mmol), paraformaldehyde (18 mmol) and cyclic secondary amine (18 mmol) were suspended in ethanol (99.8%, 20–25 ml). Glacial acetic acid (~36 mmol) was added dropwise over 0.5 h to the boiling solution until the cytosine dissolved. The resulting reaction solution was

refluxed for 3.5–4 h. After the completion of the reaction, as established by TLC ($CHCl_3$–CH_3OH, 5:1), the solvent was evaporated under vacuum and the mixture was kept under reduced pressure, on the same rotatory evaporator, at 90 °C for 1 h. After cooling, the gummy oil was shaken with benzene until a solid precipitated. Then the mixture was left in a refrigerator for 2–3 days. The precipitated solid was filtered off and crystallized from methanol. To increase the amount of precipitate, the procedure was repeated. Compound **6** was shaken with benzene and a small amount of diethyl ether. The first precipitated fraction was rejected and the procedure was repeated; only subsequent fractions were suitable for further crystallization. The spectral and analytical data of compounds **1**–**6** are given below.

Compound **1** (isolated yield 65%, mp 275–280 °C decomp.) white crystals. ^{1}H NMR (300 MHz, DMSO-d_6, ppm): δ morpholine moiety −2.31 (m, 4H, H-2,6), 3.56 (m, 4H, H-3,5); cytosine moiety −7.26 (s, 1H, H-6), 6.27 (br, 2H, NH_2), 10.46 (br, 1H, 1NH); N–CH_2–C^5 3.15 (s, 2H). ^{1}H NMR (300 MHz, TFA-*d*, ppm): δ morpholine moiety −3.58 (t, $J = 11.5$ Hz, 2H, H-2a,6a), 3.89 (d, $J = 11.9$ Hz, 2H, H-2e,6e), 4.09 (t, $J = 12.5$ Hz, 2H, H-3a,5a), 4.40 (d, $J = 12.3$ Hz, 2H, H-3e,5e); cytosine moiety −8.46 (s, 1H, H-6); N–CH_2–C^5 4.66 (s, 2H). ^{13}C NMR (75 MHz, TFA-*d*, ppm): δ morpholine moiety −63.8 (C2,6), 51.9 (C3,5); cytosine moiety −94.4 (C5), 148.1 (C2), 152.7 (C6), 159.9 (C4); N–*C*H$_2$–C^5 51.8. IR (KBr, cm^{-1}): 3428, 3290, 1664, 1654, 1617. ESI MS (*m*/*z*, %int.) 211 (100) [M+H]$^+$. Anal. Calcd for $C_9H_{14}N_4O_2$: C, 51.43; H, 6.67; N, 26.67. Found: C, 51.13; H, 6.87; N, 26.46.

Compound **2** (isolated yield 57%, mp 290–293 °C decomp.) white crystals. ^{1}H NMR (300 MHz, DMSO-d_6, ppm): δ piperidine moiety −1.48 (m, 6H, H-3,4,5), 2.49 (s, 4H, H-2,6); cytosine moiety −7.22 (s, 1H, H-6), 7.10 (br, 2H, NH_2), 10.33 (br, 1H, 1NH); N–CH_2–C^5 3.12 (s, 2H). ^{1}H NMR (300 MHz, TFA-*d*, ppm): δ piperidine moiety −1.89–2.16 (m, 6H, H-3,4,5), 3.19 (t, $J = 11.7$ Hz, 2H, H-2a,6a), 3.85 (d, $J = 10.9$ Hz, 2H, H-2e,6e); cytosine moiety −8.44 (s, 1H, H-6); N–CH_2–C^5 4.51 (s, 2H). ^{13}C NMR (75 MHz, TFA-*d*, ppm): δ piperidine moiety −20.6 (C4), 22.9 (C3,5), 54.1 (C2,6); cytosine moiety −95.2 (C5), 148.2 (C2), 152.5 (C6), 159.9 (C4); N–*C*H$_2$–C^5 51.5. IR (KBr, cm^{-1}): 3363, 3132, 1672, 1629. ESI MS (*m*/*z*, %int.) 209 (100) [M+H]$^+$. Anal. Calcd for $C_{10}H_{16}N_4O$: C, 57.69; H, 7.69; N, 26.92. Found: C, 57.46; H, 7.91; N, 26.85.

Compound **3** (isolated yield 47%, mp 283–285 °C decomp.) white crystals. ^{1}H NMR (300 MHz, TFA-*d*, ppm): δ pyrrolidine moiety −2.29 (s, 2H) and 2.38 (s, 2H): H-3,4; 3.38 (s, 2H) and 3.98 (s, 2H): H-2,5; cytosine moiety −8.44 (s, 1H, H-6); N–CH_2–C^5 4.62 (s, 2H). ^{13}C NMR (75 MHz, TFA-*d*, ppm): δ pyrrolidine moiety −22.1 (C3,4), 54.9 (C2,5); cytosine moiety −96.8 (C5), 148.5 (2), 151.6 (C6), 159.6 (C4); N–*C*H$_2$–C^5 49.2. IR (KBr, cm^{-1}): 3315, 3120, 1670, 1626. ESI MS (*m*/*z*, %int.) 195 (100) [M+H]$^+$. Anal. Calcd for $C_9H_{14}N_4O$; C, 55.67; H, 7.22; N, 28.87. Found: C, 55.63; H, 7.45; N, 28.57.

Compound **4** (isolated yield 45%, mp 253–258 ^{0}C decomp.) white crystals. ^{1}H NMR (300 MHz, DMSO-d_6, ppm): δ piperidine moiety −1.11 (t, $J = 12.3$ Hz, 2H, H-3a,5a), 1.33 (m, 1H, H-4a), 1.59 (d, $J = 12.0$ Hz, 2H, H-3e,5e), 1.82 (t, $J = 11.3$ Hz, 2H, H-2a,6a), 2.77 (d, $J = 11.3$ Hz, 2H, H-2e,6e); cytosine moiety −7.23 (s, 1H, H-6), 7.07 (br, 2H, NH_2), 10.41 (br, 1H, 1NH) N–CH_2–C^5 3.14 (s, 2H). ^{1}H NMR (300 MHz, TFA-*d*, ppm): δ piperidine moiety −1.59 (t, $J = 12.3$ Hz, 2H, H-3a,5a), 1.82 (m, 1H, H-4a), 2.10 (d, $J = 14.0$ Hz, 2H, H-3e,5e), 3.22 (t, $J = 11.8$ Hz, 2H, H-2a,6a), 3.85 (d, $J = 10.8$ Hz, 2H, H-2e,6e), 1.10 (d, $J = 5.9$ Hz, 3H, CH_3); cytosine moiety 8.44 (s, 1H, H-6); N–CH_2–C^5 4.51 (s, 2H). ^{13}C NMR (75 MHz, TFA-*d*, ppm): δ piperidine moiety −28.5 (C4), 30.9 (C3,5), 53.9 (C2,6), 19.1 (CH_3); cytosine moiety −95.3 (C5), 148.1 (C2), 152.5 (C6), 159.9 (C4); N–*C*H$_2$–C^5 51.3. IR (KBr, cm^{-1}): 3422, 3374, 1671, 1627. ESI MS (*m*/*z*, %int.) 223 (100) [M+H]$^+$. Anal. Calcd for $C_{11}H_{18}N_4O$: C, 59.46; H, 8.11; N, 25.23. Found: C, 59.26; H, 8.32; N, 25.14.

Compound **5** (isolated yield 39%, mp 263–268 °C decomp.) white crystals. ^{1}H NMR (300 MHz, TFA-*d*, ppm): δ piperidine moiety −1.16–1.35 (m, 1H, H-3a), 1.89–2.17 (m, 4H), 2.80 (t, $J = 11.7$ Hz, 1H, H-2a), 3.09 (t, $J = 10.9$ Hz, 1H, H-6a), 3.72 (d, $J = 10.5$ Hz, 1H, H-2e), 3.83 (d, $J = 10.9$ Hz, 1H, H-6e), 1.09 (d, $J = 6.3$ Hz, 3H, CH_3); cytosine moiety −8.44 (s, 1H, H-6); N–CH_2–C^5 4.51 (s, 2H). ^{13}C NMR (75 MHz, TFA-*d*, ppm): δ piperidine moiety −22.7 (C5), 29.5 (C3), 30.0 (C4), 53.4 (C6), 59.6 (C2), 16.7 (CH_3); cytosine moiety −95.5 (C5), 148.2 (C2), 152.6 (C6), 160.4 (C4); N–*C*H$_2$–C^5 51.6. IR (KBr, cm^{-1}): 3295, 3123, 1668, 1628. ESI MS (*m*/*z*, %int.) 223 (100) [M+H]$^+$. Anal. Calcd for $C_{11}H_{18}N_4O$: C, 59.46; H, 8.11; N, 25.23. Found: C, 59.36; H, 7.95; N, 25.36.

Compound **6** (isolated yield 25%, mp 205–209 °C decomp.) white crystals. ^{1}H NMR (300 MHz, TFA-*d*, ppm): δ piperidine moiety −1.35–2.20 (m, 6H), 3,16 (t, $J = 11.3$ Hz, 1H, H-2a), 3.59 (m, 1H, H-6a), 3.72 (d, $J = 11.7$ Hz, 1H, H-2e) 1.68 (d, $J = 6.3$ Hz, 3HCH$_3$); cytosine moiety 8.45 (s, 1H, H-6); N–CH_2–C^5 4.33 (s, 2H). ^{13}C NMR (75 MHz, TFA-*d*, ppm): δ piperidine moiety −21.2 (C4), 22.6 (C5), 37.1 (C3), 47.9 (C6), 64.2 (C2), 16.7 (CH_3); cytosine moiety −95.4 (C5), 148.1 (C2), 152.6 (C6), 160.2 (C4); N–*C*H$_2$–C^5 51.8. IR (KBr, cm^{-1}): 3342, 3125, 1670, 1632. ESI MS (*m*/*z*, %int.) 223 (100) [M+H]$^+$. Anal. Calcd for $C_{11}H_{18}N_4O$: C, 59.46; H, 8.11; N, 25.23. Found: C, 59.12; H, 8.09; N, 25.10.

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