Synthesis of Bis-Intercalators with an Aminochloropyrimidine Ring as Potential Anticancer Agents

Yuxiang Zhao, Hui Liang Yuan, Zuzana Zajíčková and Cyril Párkányi*

Department of Chemistry and Biochemistry, Florida Atlantic University,
777 Glades Road, P.O. Box 3091,
Boca Raton, FL 33431-0991
E-mail: parkanyi@fau.edu

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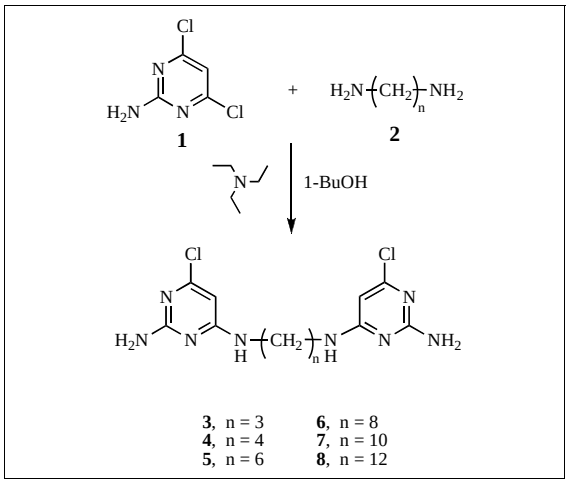

The synthesis of a series of polymethylene chain-bridged aminochloropyrimidines as potential DNA intercalators is described. *N*,*N*'-Bis(2-amino-6-chloro-4-pyrimidyl)-1,3-diaminopropane (**3**), *N*,*N*'-bis(2-amino-6-chloro-4-pyrimidyl)-1,4-diaminobutane (**4**), *N*,*N*'-bis(2-amino-6-chloro-4-pyrimidyl)-1,6-diaminohexane (**5**), *N*,*N*'-bis(2-amino-6-chloro-4-pyrimidyl)-1,8-diaminooctane (**6**), *N*,*N*'-bis(2-amino-6-chloro-4-pyrimidyl)-1,10-diaminodecane (**7**), and *N*,*N*'-bis(2-amino-6-chloro-4-pyrimidyl)-1,12-diaminododecane (**8**) were synthesized from 2-amino-4,6-dichloropyrimidine (**1**) and 1,3-diaminopropane, 1,4-diaminobutane, 1,6-diaminohexane, 1,8-diaminooctane, 1,10-diaminodecane, and 1,12-diaminododecane, respectively. The spectral data and other physical properties of the new compounds are discussed.

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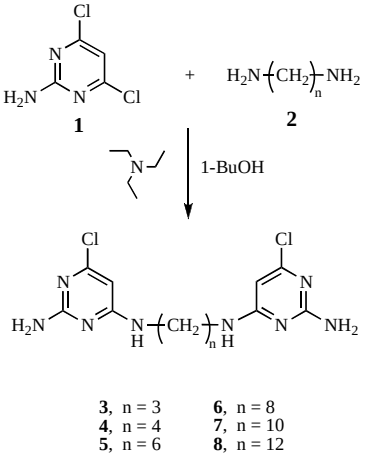
Introduction.

DNA intercalating agents are one of the most important groups of compounds that interact with the DNA double helix. Some of them are currently used for the treatment of ovarian and breast cancer and acute leukemia [1], while many others are in different phases of clinical trials. Since Lerman [2] conducted the studies about DNA intercalation in the early 1960s, extensive amount of research has been done toward the preparation and evaluation of mono- or bis-intercalating agents. Based on the well-established biological activity of compounds such as bis(9-purinyl)ethane and the ethylenediamine derivatives [3-6], and within the framework of our systematic studies of novel heterocyclic compounds with potential biological activity [7-16], we have synthesized another series of bis-aminochloropyrimidine compounds bridged by polymethylene chains of different length.

Results and Discussion.

In the present paper, we report the preparation of *N*,*N*'-bis(2-amino-6-chloro-4-pyrimidyl)-1,3-diaminopropane (**3**), *N*,*N*'-bis(2-amino-6-chloro-4-pyrimidyl)-1,4-diaminobutane (**4**), *N*,*N*'-bis(2-amino-6-chloro-4-pyrimidyl)-1,6-diaminohexane (**5**), *N*,*N*'-bis(2-amino-6-chloro-4-pyrimidyl)-1,8-diaminooctane (**6**), *N*,*N*'-bis(2-amino-6-chloro-4-pyrimidyl)-1,10-diaminodecane (**7**), and *N*,*N*'-bis(2-amino-6-chloro-4-pyrimidyl)-1,12-diaminododecane (**8**). The compounds were synthesized by nucleophilic substitution of 2-amino-4,6-dichloropyrimidine (**1**) with the appropriate aliphatic diamines (**2**): 1,3-diaminopropane, 1,4-diaminobutane, 1,6-diaminohexane, 1,8-diaminooctane, 1,10-diaminodecane, and 1,12-diaminododecane, respectively, in the presence of triethylamine by refluxing in 1-butanol as the solvent (Scheme 1). Similar methods have been described before [17-19] with different starting materials. Compared to the procedure with 5-amino-4,6-dichloropyrimidine established previously [16], this series of reactants gives much better yields, from 73% to 87%, based on our experimental data. The possible reason is the different position of the amino group in 2-amino-4,6-dichloropyrimidine (**1**).

Scheme 1



3, n = 3 **6**, n = 8
4, n = 4 **7**, n = 10
5, n = 6 **8**, n = 12

The structures of these new compounds were determined on the basis of their analytical and spectral data, which are in agreement with their proposed structures. For example, the infrared spectra of this series of synthesized compounds have the characteristic N-H bond stretching absorptions in the 3300-3500 cm^{-1} region due to the presence of primary and secondary amino groups. Aromatic C=N stretching has absorptions around 1640 cm^{-1}, whereas the aliphatic C-N stretching is observed in the 1159-1170 cm^{-1} region. The C-Cl stretching absorption frequency is observed around 780 cm^{-1}. All the compounds have an almost identical pattern of UV-vis spectra because of their structure.

The ^1H NMR spectra of the new synthesized compounds show all the expected peaks corresponding to proposed structures. The primary and secondary amino group proton signals appear in the 6.36-6.42 ppm range and 7.08-7.16 ppm range, respectively. The proton on the aromatic pyrimidine ring gives a signal at 5.70-5.75 ppm. All ^{13}C NMR spectra were measured in dimethyl sulfoxide-d_6 (DMSO-d_6) because of poor solubility of the products. However, in almost all ^{13}C spectra, signal of the carbon next to the secondary amine on the polymethylene chain was missing. To study this problem, we obtained a ^{13}C spectrum of **5** in CD_3OD to compare with the spectrum in DMSO-d_6. With CD_3OD as the solvent, one more peak appears at 40.25 ppm, which is close to the region of DMSO-d_6 solvent signal at 40.45 ppm [20] in ^{13}C NMR. Therefore, the reason for the missing signal of that methylene carbon could be an overlap with DMSO-d_6 solvent peak.

The new compounds will be tested for their biological activity as potential DNA intercalating agents at the National Cancer Institute.

EXPERIMENTAL

The melting points were determined on the Fisher-Johns melting point apparatus (W.H. Curtin & Co.). ^1H and ^{13}C NMR spectra were recorded with a Varian 300-MHz spectrometer. Infrared spectra were measured on a 4020 GALAXY series FT-IR spectrometer (Mattson Instruments) (potassium bromide disk). Mass spectra were recorded on an M-8000 Hitachi mass spectrometer with an L-7100 pump and ion trap mass analyzer. All mass spectra were obtained in ESI positive mode. UV spectra were measured on a Cary 3 UV-visible spectrophotometer. Thin layer chromatography (TLC) of samples used silica gel 60 F-254 precoated plates and the spots were located in the UV light or by iodine vapor. Elemental analyses were performed by Desert Analytics, Tucson, AZ, and by Atlantic Microlab Inc., Norcross, GA. All solvents used were reagent grade, except for the dimethyl sulfoxide and methanol in NMR spectroscopic measurements.

N,N'-Bis(2-amino-6-chloro-4-pyrimidyl)-1,3-diaminopropane (**3**).

2-Amino-4,6-dichloropyrimidine (1.64 g, 0.01 mol) and 0.37 g (0.005 mol) 1,3-diaminopropane was suspended in 30 ml of 1-butanol and 1.2 g (0.012 mol) of triethylamine was added dropwise. The reaction mixture was refluxed for 6 hours under argon. Then the solvent was evaporated under vacuum. The light yellow solid residue was recrystalized from methylene chloride-ethanol (1: 2 vol.), 1.25 g of beige product was collected, yield 76%. mp: 228-230 °C. ^1H NMR (DMSO-d_6): 7.16 (s, 2NH), 6.42 (s, $2NH_2$), 5.75 (s, 2CH), 3.25 (s, $2CH_2$), 1.66-1.71 (m, CH_2) ppm. ^{13}C NMR (DMSO-d_6): 164.05, 162.99, 157.22, 92.76, 37.66, 28.72 ppm. IR (KBr): 3467 (m, NH_2), 3376 (m, N-H), 2975 (m, CH_2), 2850 (m, CH_2), 1648 (s, C=N), 1579 (s, NH_2 bending), 1483 (s, N-H bending), 1160 (s, C-N), 780 (m, C-Cl) cm^{-1}. UV (methanol) λ_{max} (log ε): 286 nm (4.48), 238 nm (4.57), 212 nm (4.92). MS m/z: 329.25 M^+.

Anal. Calcd. for $C_{11}H_{14}Cl_2N_8$: C, 40.13; H, 4.29; Cl, 21.54; N, 34.04. Found: C, 40.42; H, 4.43; Cl, 21.37; N, 33.81.

N,N'-Bis(2-amino-6-chloro-4-pyrimidyl)-1,4-diaminobutane (**4**).

2-Amino-4,6-dichloropyrimidine (1.64 g, 0.01 mol) and 0.44 g (0.005 mol) 1,4-diaminobutane was suspended in 30 ml of 1-butanol and 1.2 g (0.012 mol) of triethyamine was added dropwise. The reaction mixture was refluxed for 6 hours under argon. Then the solvent was evaporated under vacuum. The beige solid residue was recrystalized from 2-propanol-acetone-water (5:5:1 vol.), 1.33 g of off-white solid was collected after two recrystalizations, yield 76%. m.p.: 213-215 °C. ^1H NMR (DMSO-d_6): 7.13 (s, 2NH), 6.38 (s, $2NH_2$), 5.71 (s, 2CH), 3.23 (s, $2CH_2$), 1.45-1.50 (m, $2CH_2$) ppm. ^{13}C NMR (DMSO-d_6): 164.01, 163.00, 157.13, 92.71, 26.38 ppm. IR (KBr): 3326 (m, NH_2), 3135 (m, N-H), 2933 (m, CH_2), 2869 (m, CH_2), 1662 (s, C=N), 1587 (s, NH_2 bending), 1473 (s, N-H bending), 1159 (m, C-N), 780 (m, C-Cl) cm^{-1}. UV (methanol) λ_{max} (log ε): 284 (4.46), 239 (4.55), 212 nm (4.94). MS m/z: 343.19 M^+.

Anal. Calcd. for $C_{12}H_{16}Cl_2N_8$: C, 41.99; H, 4.70. Found: C, 41.86; H, 4.72.

N,N'-Bis(2-amino-6-chloro-4-pyrimidyl)-1,6-diaminohexane (**5**).

2-Amino-4,6-dichloropyrimidine (1.64 g, 0.01 mol) and 0.68 g (0.005 mol) 1,6-diaminohexane was suspended in 30 ml of 1-butanol and 1.2 g (0.012 mol) of triethylamine was added

dropwise. The reaction mixture was refluxed for 8 hours under argon. Then the solvent was evaporated under vacuum. The beige solid residue was recrystalized from 2-propanol first, 1.62 g of white solid was collected, yield 87%. The second recrystalization was carried out from 2-propanol-acetone (6:4 vol.), mp: 214-216 °C. ^{1}H NMR (DMSO-d_6): 7.10 (s, 2NH), 6.38 (s, 2NH_2), 5.70 (s, 2CH), 3.20 (s, 2CH_2), 1.42-1.47 (m, 2CH_2), 1.29 (s, 2CH_2) ppm. ^{13}C NMR (DMSO-d_6): 164.01, 162.95, 157.03, 92.70, 28.89, 26.29 ppm. ^{13}C NMR (CD_3OD): 164.60, 93.78, 40.29, 29.09, 26.53 ppm. IR (KBr): 3502 (m, NH_2), 3268 (m, N-H), 3153 (m), 2935 (m, CH_2), 2854 (m, CH_2), 1598 (s, C=N), 1444 (s, NH_2 bending), 1368 (s, N-H bending), 1170 (m, C-N), 786 (m, C-Cl) cm^{-1}. UV (methanol) λ_{max} (log ε): 284 (4.45), 240 (4.54), 213 nm (4.93). MS m/z: 371.26 M$^+$.

Anal. Calcd. for $C_{14}H_{20}Cl_2N_8$: C, 45.29; H, 5.43; N, 30.18. Found: C, 45.37; H, 5.30; N, 29.76.

N,N'-Bis(2-amino-6-chloro-4-pyrimidyl)-1,8-diaminooctane (**6**).

2-Amino-4,6-dichloropyrimidine (1.64 g, 0.01 mol) and 0.77 g (0.005 mol) 1,8-diaminooctane was suspended in 30 ml of 1-butanol and 1.2 g (0.012 mol) of triethylamine was added dropwise. The reaction mixture was refluxed for 10 hours. Then the solvent was evaporated under vacuum. The light yellow solid residue was recrystalized from ethanol-water (6:1 vol.), 1.65 g of white solid was collected, yield 82%. mp: 216-218 °C. ^{1}H NMR (DMSO-d_6): 7.10 (s, 2NH), 6.38 (s, 2NH_2), 5.71 (s, 2CH), 3.20 (s, 2CH_2), 1.42-1.47 (m, 2CH_2), 1.27(s, 2$(CH_2)_2$) ppm. ^{13}C NMR (DMSO-d_6): 164.03, 163.02, 157.1, 92.65, 28.88, 26.49 ppm. IR (KBr): 3428 (m, NH_2), 3323 (m, N-H), 3178 (m), 2932 (m, CH_2), 2856 (m, CH_2), 1646 (s, C=N), 1585 (s, NH_2 bending), 1461 (s, N-H bending), 1165 (m, C-N), 786 (m, C-Cl) cm^{-1}. UV (methanol) λ_{max} (log ε): 285 nm (4.47), 238 nm (4.57), 213 nm (4.96). MS m/z: 399.52 M$^+$.

Anal. Calcd. for $C_{16}H_{24}Cl_2N_8$: C, 48.12; H, 6.06; Cl, 17.76. Found: C, 48.27; H, 6.28; Cl, 17.75.

N,N'-Bis(2-amino-6-chloro-4-pyrimidyl)-1,10-diaminodecane (**7**).

2-Amino-4,6-dichloropyrimidine (1.64 g, 0.01 mol) and 0.86 g (0.005 mol) 1,10-diaminodecane was suspended in 30 ml of 1-butanol and 1.2 g (0.012 mol) of triethylamine was added dropwise. The reaction mixture was refluxed for 12 hours. Then the solvent was evaporated under vacuum. 1.59 g of beige solid was first recrystalized from ethanol, yield 74%. The second recrystalization was from ethanol-acetone (8:2 vol.), mp: 167-168 °C. ^{1}H NMR (DMSO-d_6): 7.08 (s, 2NH), 6.36 (s, 2NH_2), 5.70 (s, 2CH), 3.20 (s, 2CH_2), 1.42-1.45 (m, 2CH_2), 1.24 (s, 2$(CH_2)_3$) ppm. ^{13}C NMR (DMSO-d_6): 163.94, 162.88, 156.97, 92.70, 29.05, 28.86, 26.50 ppm. IR (KBr): 3402 (m, NH_2), 3171 (m, N-H), 2926 (m, CH_2), 2852 (m, CH_2), 1640 (s, C=N), 1582 (s, NH_2 bending), 1466 (s, NH bending), 1161 (m, C-N), 786 (m, C-Cl) cm^{-1}. UV (methanol) λ_{max} (log ε): 285 (4.48), 239 (4.59), 213 nm (4.98). MS m/z: 427.48 M$^+$.

Anal. Calcd. for $C_{18}H_{28}Cl_2N_8$: C, 50.59; H, 6.60. Found: C, 50.46; H, 6.61.

N,N'-Bis(2-amino-6-chloro-4-pyrimidyl)-1,12-diaminododecane (**8**).

2-Amino-4,6-dichloropyrimidine (1.64 g, 0.01 mol) and 1 g (0.005 mol) 1,12-diaminododecane was suspended in 30 ml of 1-butanol and 1.2 g (0.012 mol) of triethylamine was added dropwise. The reaction mixture was refluxed for 12 hours. Then the solvent was evaporated under vacuum. A beige solid was recrystalized from ethanol-methanol (9:1 vol.), and 1.66 g of product was collected, yield 73%. The second recrystalization was from dichloromethane-acetone-ethyl acetate (1:1:1 vol.), mp: 78-80 °C. ^{1}H NMR (DMSO-d_6): 7.13 (s, 2NH), 6.40 (s, 2NH_2), 5.71 (s, 2CH), 3.20 (s, 2CH_2), 1.42-1.47 (m, 2CH_2), 1.24 (s, 2$(CH_2)_4$) ppm. ^{13}C NMR (DMSO-d_6): 163.94, 162.85, 156.91, 92.73, 29.10, 29.07, 28.87, 26.51 ppm. IR (KBr): 3305 (m, NH_2), 3166 (m, N-H), 2924 (m, CH_2), 2851 (m, CH_2), 1586 (s, NH_2 bending), 1464 (s, NH bending), 1159 (m, C-N), 780 (m, C-Cl) cm^{-1}. UV (methanol) λ_{max} (log ε): 285 (4.47), 239 (4.57), 213 nm (4.97). MS m/z: 427.48 M$^+$, 228.18.

Anal. Calcd. for $C_{20}H_{32}Cl_2N_8$: C, 52.74; H, 7.08; Cl, 15.57. Found: C, 52.86; H, 7.17; Cl, 15.97.

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