Dedicated to Prof. Sorin Rosca on the occasion of his 70th birthday

Synthesis of 4,6-Disubstituted Dihydrodipyridopyrazines and Assessment of Their DNA Binding

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4,6-Disubstituted dihydrodipyridopyrazines have been synthesized and evaluated as antitumor agents. An investigation of their DNA binding capacities was performing. Compared to 4-monosubstituted dihydrodipyridopyrazines, corresponding disubstituted dihydrodipyridopyrazines showed lower affinity for DNA. Additional studies for their cytotoxic properties are underway.

Keywords: metalation, reductive amination, DNA binding, melting temperature measurements, absorption measurements

Recently, we developed dihydrodipyridopyrazines [1,2] as a new family of potential antitumor agents. By analogy with related structures (e.g., phenazine and acridines), these tricyclic planar molecules are thought to intercalate into DNA. Aminoalkyl-substituted monomeric and dimeric dihydrodipyridopyrazines were identified as potent cytotoxic compounds. Biochemical and biophysical studies indicated that all these compunds strongly stabilized the duplex structure of DNA and some of them elicited selectivity for GC-rich sequences [3].

To extend the structure-activity relationships [4] in this series, we report here the synthesis of some 4,6-disubstituted dihydrodipyridopyrazines together with information on their DNA binding capacities.

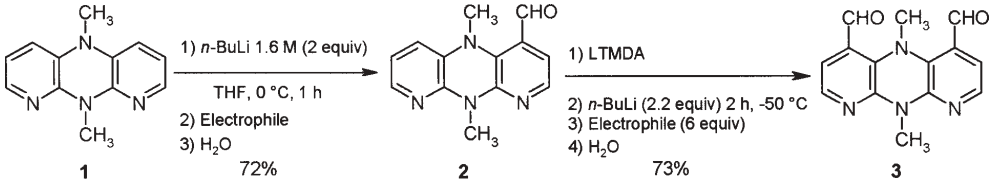
Synthesis of 4,6-Disubstituted Dihydrodipyridopyrazines

The procedure for synthesis of aldehyde **3** (scheme 1) has been previously described [5].

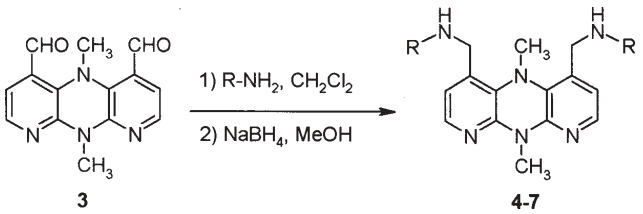
Briefly, when **1** was submitted to lithiation with *n*-butyllithium in tetrahydrofuran, after addition of *N*,*N*-dimethylformamide and workup, 5,10-dimethyl-5,10-dihydrodipyrido-pyrazines-4-carbaldehyde **2** was isolated in 72%. We performed then the lithiation reaction directed by amino alkoxides using *N*,*N*,*N*'-trimethylethylenediamine as the amine component, 2,2 equiv of *n*-butyl lithium as metalation agent at –50°C, and 6 equiv of *N*,*N*-dimethylformamide as electrophile. Lithiation occurred on 6-position and allowed us easy acces to 4,6-dicarbaldehyde **3** in 73% yield.

Considering that the incorporation of an aminoalkyl substituent generally enhances the cytotoxic potential of DNA binding agents it was then decided to introduce such aminogroups on **3** as depicted in scheme 2 and table 1. Compounds **4-7** were obtained in good yields by classical reductive amination.

For a comparative study with the disubstituted analogue we also synthesized the 4-monosubstituted dihydrodipyridopyrazine **8** (scheme 3).

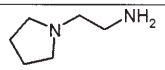
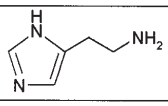


Scheme 1



Scheme 2

Table 1

Entry	R-NH_2	Product	Yield (%)
1	$Me_2N(CH_2)_2NH_2$	**4**	59
2	$Me_2N(CH_2)_3NH_2$	**5**	61
3		**6**	51
4		**7**	52

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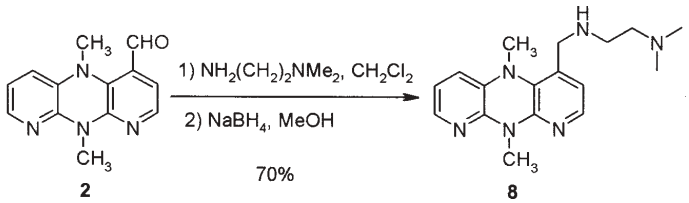

Scheme 3

DNA Binding

The disubstituted dihydrodipyridopyrazines **4-7** and the monosubstituted dihydrodipyridopyrazines **8** were submitted for an investigation of their DNA binding capacities.

Two complementary technical approaches were deployed to evaluate their affinity for DNA melting temperature (T_m) measurements and absorption measurements.

The results of T_m analysis performed with CTDNA and poly(dAT2) are shown in figure 1. The ΔT_m values ($\Delta T_m = T_m^{complex} - T_m^{DNA}$) are compared. We observed that the monosubstituted dihydrodipyridopyrazine **8** presents the higher ΔT_m value (9°C) comparable to those observed with the disubstituted dihydrodipyridopyrazines **4-7**, which proved that the stabilizing duplex DNA against heat denaturation exits but is not very efficient (see the ΔT_m values for some dimers which are 20-25°C) [3].

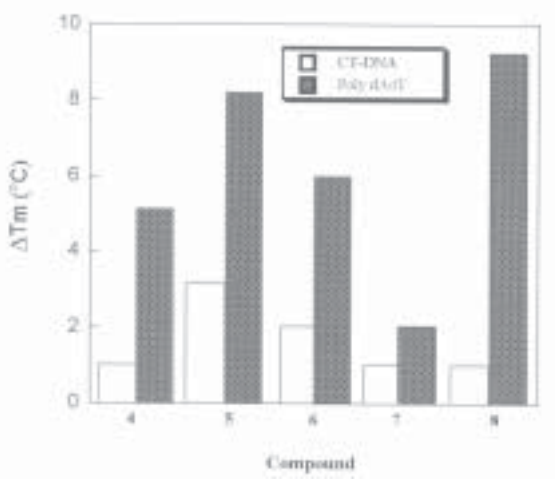

Fig. 1. Variation of ΔTm ($T_m^{drug\text{-}DNAcomplex} - T_m^{DNAalone}$, in Celsius degrees) of the complexes between the test compounds and calf thymus DNA (black bars) or poly(dAT)$_2$ (white bars).

The differences observed between the monosubstituted compound **8** and the disubstituted compounds **4-7** was also evident from absorption measurements. The higher hypochromic and bathocromic shifts were observed also in the case of **8** (13 nm) (fig. 2). The disubstituted compounds **4-7** showed low values of hypochromic and bathocromic shifts (3-6 nm).

Experimental Section

Chemistry - Materials and Methods: Mps were determined on a Tottoli or a Kofler melting point apparatus and are uncorrected. ^{1}H and ^{13}C NMR spectra were recorded in $CDCl_3$ with a Bruker 250 MHz spectrometer (Attached Proton Test Method, APT) or a Bruker instrument Avance DPX 250 at 250.131 and 62.9 MHz, respectively. Chemical Shifts (δ values) were reported in parts per million and coupling constants (*J* values) in Hz. TMS was the internal standard. Infrared spectra were recorded using NaCl films or KBr pellets techniques on a Perkin-Elmer 841 instrument. Mass spectra (MS) were recorded on a Perkin-Elmer mass spectrometer SCIEX API 300 by ion spray (IS).TLC was performed with plates coated with Kieselgel G (Merck). The silica gel used for flash chromatography was Kieselgel of 0.04-0.063 mm particle size. Reagent-grade THF was first distilled from potassium hydroxide, then from sodium benzophenone ketyl and stored over sodium until used.

Reductive Amination of 5,10-dimethyl-5,10-dihydrodipyridopyrazines-4,6-carbaldehyde 3: Preparation of Compounds 4-7. A typical procedure is exemplified by the preparation of compound **4**. To a solution of 0.18 mmol (50 mg, 1 equiv) of **3** in 5 mL of methylene chloride was added under argon atmosphere 1.1 mmol (121μL, 6 equiv) of N,N-dimethylethylenediamine. After stirring for 2 h at room temperature, the solvent was removed under vacuum. The residue was dissolved in 5 mL of MeOH and1.8 mmol (70 mg, 10 equiv) of $NaBH_4$ was added. After stirring for 1 h at room temperature, the reaction medium

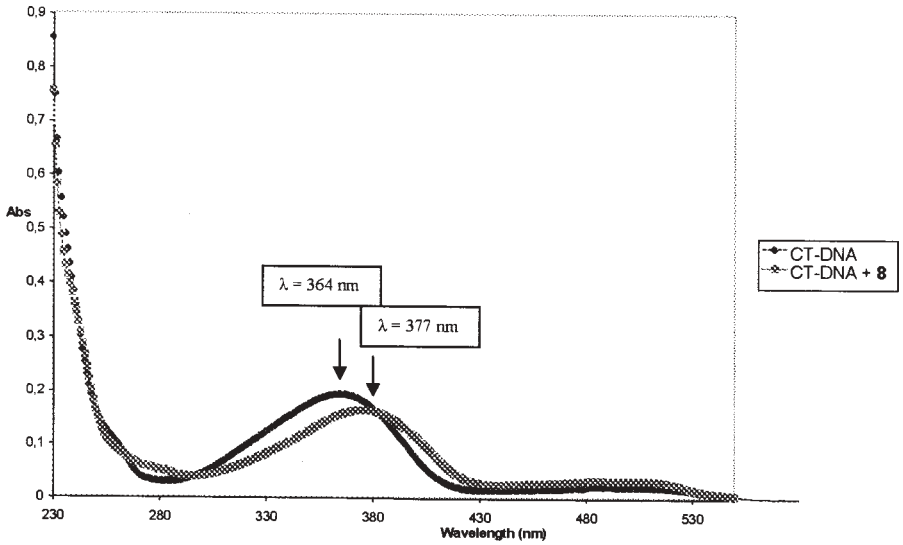

Fig. 2 Absorption measurements for compound 8

was hydrolyzed at 0 °C and extracted with methylene chloride. The organic layer was dried over $MgSO_4$. The compound **4** was obtained as a gum in 59% yield after column chromatography (CH_2Cl_2 / MeOH / NH_4OH : 100 / 10 / 1)

Compound **4**: IR (NaCl): 3381 cm^{-1}(NH); MS: m/z = 413.5 (M+1); ^1H NMR ($CDCl_3$, 250 MHz): δ = 1.99 (sl, 2H, 2N**H**); 2.16 (s, 12H, 4C**H**$_3$, 2NMe_2); 2.38 (t, 4H, 2C**H**$_2$NMe_2, *J* = 5.9 Hz); 2.63 (q, 4H, 2HNC**H**$_2$, *J* = 5.9 Hz, *J* = 11.0 Hz); 2.88 (s, 3H, C**H**$_3$); 3.53 (s, 3H, C**H**$_3$); 3.70 (d, 2H, C**H**$_2$NH, *J* = 14.8 Hz); 3.98 (d, 2H, C**H**$_2$NH, *J* = 14.5 Hz); 6.89 (d, 2H, **H**$_3$, **H**$_7$, *J* = 5.1 Hz); 7.93 (d, 2H, **H**$_2$, **H**$_8$, *J* = 5.1 Hz); ^{13}C NMR ($CDCl_3$, 62.9 MHz): δ = 28.7 (**C**H_3); 45.6 (4**C**H_3, 2NMe_2); 46.5 (**C**H_3); 46.9 (2**C**H_2); 48.0 (2**C**H_2); 59.2 (2**C**H_2); 117.1 (2CH, **C**$_3$, **C**$_7$); 130.3 (2C, **C**$_4$, **C**$_6$); 142.0 (2C, **C**$_{4a}$, **C**$_{5a}$); 143.3 (2CH, **C**$_2$, **C**$_8$); 152.7 (2C, **C**$_{9a}$, **C**$_{10a}$).

Compound **5**: IR (NaCl): 1404, 1640, 3410 cm^{-1}(NH); MS: m/z = 441.6 (M+1); ^1H NMR ($CDCl_3$, 250 MHz): δ = 1.62-1.73 (m, 4H, 2C**H**$_2$); 2.20 (s, 12H, 4C**H**$_3$, NMe_2); 2.29 (t, 4H, 2C**H**$_2$NMe_2, *J* = 7.2 Hz); 2.47 (sl, 2H, 2N**H**); 2.64-2.69 (m, 4H, 2C**H**$_2$); 2.86 (s, 3H, C**H**$_3$); 3.51 (s, 3H, C**H**$_3$); 3.67 (d, 2H, C**H**$_2$NH, *J* = 14.5 Hz); 3.94 (d, 2H, C**H**$_2$NH, *J* = 14.5 Hz); 6.87 (d, 2H, **H**$_3$, **H**$_7$, *J* = 5.1 Hz); 7.92 (d, 2H, **H**$_2$, **H**$_8$, *J* = 5.1 Hz); ^{13}C NMR ($CDCl_3$, 62.9 MHz): δ = 27.8 (2**C**H_2); 28.7 (**C**H_3); 45.5 (4**C**H_3, NMe_2), 46.4 (**C**H_3); 48.0 (2**C**H_2); 48.2 (2**C**H_2N); 58.1 (2**C**H_2N); 116.9 (2CH, **C**$_3$, **C**$_7$); 130.2 (2C, **C**$_4$, **C**$_6$); 141.5 (2C, **C**$_{4a}$, **C**$_{5a}$); 143.4 (2CH, **C**$_2$, **C**$_8$); 152.7 (2C, **C**$_{9a}$, **C**$_{10a}$).

Compound **6**: IR (NaCl): 3380 cm^{-1}(NH); MS: m/z = 465.5 (M+1); ^1H NMR ($CDCl_3$, 250 MHz): δ = 1.70-1.75 (m, 8H, 4C**H**$_2$); 2.01 (sl, 2H, 2N**H**); 2.41-2.44 (m, 8H, 4C**H**$_2$); 2.57-2.62 (m, 4H, 2C**H**$_2$); 2.68-2.73 (m, 2H, 2C**H**$_2$); 2.88 (s, 3H, C**H**$_3$); 3.53 (s, 3H, C**H**$_3$); 3.70 (d, 2H, C**H**$_2$NH, *J* = 14.6 Hz); 3.99 (d, 2H, C**H**$_2$NH, *J* = 14.6 Hz); 6.89 (d, 2H, **H**$_3$, **H**$_7$, *J* = 5.1 Hz); 7.93 (d, 2H, **H**$_2$, **H**$_8$, *J* = 5.1 Hz); ^{13}C NMR ($CDCl_3$, 62.9 MHz): δ = 23.5 (4C**H**$_2$); 28.7 (C**H**$_3$); 46.4 (C**H**$_3$); 48.0 (2C**H**$_2$); 48.1 (2C**H**$_2$); 54.3 (4C**H**$_2$); 55.9 (2C**H**$_2$); 117.2 (2CH, **C**$_3$, **C**$_7$); 130.3 (2C, **C**$_4$, **C**$_6$); 141.9 (2C, **C**$_{4a}$, **C**$_{5a}$); 143.4 (2CH, **C**$_2$, **C**$_8$); 152.7 (2C, **C**$_{9a}$, **C**$_{10a}$).

Compound **7**: IR (NaCl): 3416 cm^{-1}(NH); MS: m/z = 459.5 (M+1); ^1H NMR ($CDCl_3$, 250 MHz): δ = 2.76 (t, 4H, 2C**H**$_2$, *J* = 6.7 Hz); 2.83 (s, 3H, C**H**$_3$); 2.86-2.93 (m, 4H, 2C**H**$_2$); 3.46 (s, 3H, C**H**$_3$); 3.75 (d, 2H, C**H**$_2$NH, *J* = 14.2 Hz); 4.06 (d, 2H, 2C**H**$_2$NH, *J* = 14.2 Hz); 6.78 (s, 2H, 2C**H**); 6.93 (d, 2H, **H**$_3$, **H**$_7$, *J* = 5.1 Hz); 7.51 (s, 1H, C**H**); 7.90 (d, 2H, **H**$_2$, **H**$_8$, *J* = 5.1 Hz); ^{13}C NMR ($CDCl_3$, 62.9 MHz): δ = 27.3 (2**C**H_2); 29.1 (**C**H_3); 46.9 (**C**H_3); 47.8 (2**C**H_2); 49.7 (2**C**H_2); 117.4 (2**C**H); 118.6 (2CH, **C**$_3$, **C**$_7$); 131.5 (2C, **C**$_4$, **C**$_6$); 136.1 (2**C**H); 136.3 (2C); 142.2 (2C, **C**$_{4a}$, **C**$_{5a}$); 144.6 (2CH, **C**$_2$, **C**$_8$); 153.8 (2C, **C**$_{9a}$, **C**$_{10a}$).

Preparation of Compound **8**. The same procedure was applied for the preparation of **8** using the 5,10-dimethyl-5,10-dihydrodipyridopyrazines-4-carbaldehyde **2.**

Compound **8**: IR (NaCl): 3417 cm^{-1}(NH); MS: m/z = 313 (M+1); ^1H NMR ($CDCl_3$, 250 MHz): δ = 2.18 (s, 6H, 2C**H**$_3$, NMe_2); 2.38 (t, 2H, C**H**$_2$, *J* = 6.2 Hz); 2.62 (t, 2H, C**H**$_2$, *J* = 6.2 Hz); 3.19 (s, 3H, NC**H**$_3$); 3.38 (s, 3H, NC**H**$_3$); 3.68 (s, 2H, C**H**$_2$NH); 6.57 (dd, 1H, **H**$_6$, *J* = 4.9 Hz, *J* = 7.5 Hz); 6.65-6.69 (m, 2H, **H**$_3$, **H**$_7$); 7.64-7.68 (m, 2H, **H**$_2$, **H**$_8$); ^{13}C NMR ($CDCl_3$, 62.9 MHz): δ = 28.3 (N**C**H_3); 44.6 (N**C**H_3); 45.4 (2**C**H_3, NMe_2); 46.5, 49.5 (2**C**H_2); 58.9 (**C**H_2NH); 116.8, 118.4, 122.2 (3CH, **C**$_3$, **C**$_6$, **C**$_7$); 130.5, 134.7, 135.5 (3C, **C**$_4$, **C**$_{4a}$, **C**$_{5a}$); 140.6, 140.8 (2CH, **C**$_2$, **C**$_8$); 150.7, 151.8 (2C, **C**$_{9a}$, **C**$_{10a}$).

Molecular Pharmacology. The experimental procedures used for the melting temperature and absorption measurements have been previously described [6].

Conclusions

In conclusion, we have synthesized new 4,6-disubstituted dihydrodipyridopyrazines and we have investigated their DNA binding capacities. These compounds present a weak effect. Additional studies for their cytotoxic properties are underway, and the results will be reported in due course.

References

1. (a) BLANCHARD, S., RODRIGUEZ, I., KUEHM-CAUBÈRE, C., RENARD, P., PFEIFFER, B., GUILLAUMET, G., CAUBÈRE, P., Tetrahedron 2002, 58, 3513. (b) BLANCHARD, S., RODRIGUEZ, I., CAUBÈRE, P., GUILLAUMET, G. Synlett, 8, 2002, p. 1356

2. (a) GRIG-ALEXA, I. C., FINARU, A. L., IVAN, L., CAUBÈRE, P., GUILLAUMET, G., Tetrahedron Lett. 2004, 45, 2343. (b) GRIG-ALEXA, I. C., FINARU, A. L., IVAN, L., CAUBÈRE, P., GUILLAUMET, G. Synthesis, 4, 2006, p. 619

3. (a) CAUBÈRE, P., GUILLAUMET, G.; RODRIGUEZ, I., VINTER-PASQUIER, K., KUEHM-CAUBÈRE, C., BLANCHARD, S., ATASSI, G., PIERRE, A., PFEIFFER, B., RENARD, P. P. Eur. Pat. Appl. EP 96986, 1999, Chem. Abstr. 2000, 132, 22978. (b) BLANCHARD, S., RODRIGUEZ, I.,M TARDY, C., BALDEYROU, B., BAILLY, C., COLSON, P., HOUSSIER, C., LÉONCE, S., KRAUS-BERTHIER, L., PFEIFFER, B., RENARD, P., PIERRE, A., CAUBÈRE, P., GUILLAUMET, G. J. Med. Chem., **47**, 2004, p. 978

4. LASZLO, T,. Rev. Chim. (Bucure°ti), **58**, nr. 7, 2007, p. 632

5. GRIG-ALEXA, I. C., FINARU, A. L., CAUBÈRE, P., GUILLAUMET, G. Organic Letters, 2006, 19, p. 4187

6. (a) COLSON, P., BAILLY, C., HOUSSIER, C. Biophys. Chem. 1996, 58, 125. (b) GOOSENS, J.F., BOUEY-BENCTEUX, E., HOUSSIN, R., HENICHART, J.P., COLSON, P., HOUSSIER, C., LAINE, W., BALDEYROU, B., BAILLY, C. Biochemistry 40, 2001, p. 4663

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