# SYNTHESIS, INTERCALATION INTO DNA AND ANTICANCER ACTIVITY OF SOME 4,6-DIALKOXY-10-METHYL-PYRIDO [3,2-g] QUINOLINES

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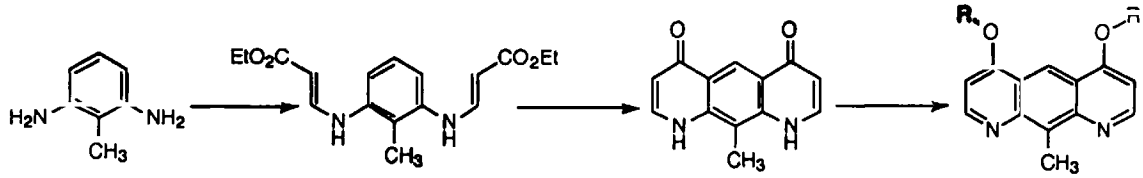
**ABSTRACT** : New derivatives known as 4,6-dialkoxy-10-methyl-pyrido[3,2-g] quinolines, were prepared with a view to be tested as anticancer agents. In addition, intercalation of these ligands into DNA was spectrophotometrically measured.

## INTRODUCTION

We were interested in the pyridoquinoline diones series with a view to prepare anticancer agents, owing to the biological activity of related compounds such as diazaquinomycin [1] or 10-hydroxy-1,4,6,9-tetramethyl-pyrido[3,2-g]quinoline-2,8-(1H,9H)-dione [2].

## RESULTS AND DISCUSSION

The 10-methyl pyrido[3,2-g]quinoline-4,6-dione was selected as starting compound. This compound was prepared according to the method previously reported [3]. Alkylation was achieved under phase transfer catalysis conditions with toluene as solvent and tetrabutylammonium bromide (TEBAB) as catalyst (scheme 1).




scheme 1 : synthetic pathway

Chemical data about the dialkoxy derivatives prepared, are presented in Table 1.

Table 1 : Chemical data*

| Cmpd** | R | Time (hrs) | Yield (%) | mp (°C) | $^1$H NMR ($CDCl_3$/TMS)*** - δ (ppm) - J (Hz) |
|---|---|---|---|---|---|
| **1a** | $-CH_2CH_3$ | 24 | 74 | 210-212 | 1.65(t, J = 7.0 Hz, 6H, $CH_3$) ; 3.35(s, 3H, $CH_3$) ; 4.30(q, J = 7.0 Hz, 4H, O-$CH_2$) ; 6.65(d, J = 5.0 Hz, 2H, Ar) ; 8.85(d, J = 5.0 Hz, 2H, Ar) ; 9.00(s, 1H, Ar) |
| **1b** | $-CH(CH_3)_2$ | 24 | 52 | 206-208 | 1.5(d, J = 6.0 Hz, 12H,$CH_3$) ; 3.35(s, 3H, $CH_3$) ; 4.90(spt, J = 6.0 Hz, 2H, CH) ; 6.65(d, J = 5.0 Hz, 2H, Ar) ; 8.90(d, J = 5.0 Hz, 2H, Ar) ; 9.05(s, 1H, Ar) |
| **1c** | $-(CH_2)_2-N(CH_3)_2$ | 24 | 20 | 188-190 | 2.95(s, 12H, N-$CH_3$) ; 3.00(s, 3H, $CH_3$) ; 3.85(t, J = 4.5 Hz, 4H, N-$CH_2$) ; 4.90(t, J = 4.5 Hz, 4H, O-$CH_2$) ; 7.45(d, J = 6.7 Hz, 2H, Ar) ; 9.10(d, J = 6.7 Hz, 2H, Ar) ; 9.50(s, 1H, Ar) |
| **1d** | $-(CH_2)_2-N(C_2H_5)_2$ | 36 | 39 | 145-148 | 1.15(t, J = 7.1 Hz, 12H, $CH_3$) ; 2.75(q, J = 7.1 Hz 8H, N-$CH_2$) ; 3.10(t, J = 6.1 Hz, 4H, $CH_2$-N) ; 3.35(s,3H) ; 4.30(t, J = 6.1 Hz, 4H, O-$CH_2$) ; 6.70(d, J = 4.9 Hz 2H, Ar) ; 8.90(d, J = 4.9 Hz, 2H, Ar) ; 9.05(s, 1H, Ar) |
| **1e** | $-(CH_2)_3-N(CH_3)_2$ | 36 | 20 | 136-138 | 2.20(m, 4H, $CH_2$) ; 2.30(s, 12H, N-$CH_3$) ; 2.60(t, J = 7.1 Hz, 4H, $CH_2$-N) ; 3.35(s, 3H, $CH_3$) ;4.35(t, J = 4.9 Hz, 4H, O-$CH_2$) ; 6.70(d, J = 4.9 Hz, 2H, Ar) ; 8.90(d, J = 4.9 Hz, 2H, Ar) ; 9.00(s, 1H, Ar) |
| **1f** | $-(CH_2)_2-N$  | 48 | 20 | 140-142 | 1.85(m, 8H, $CH_2$) ; 2.75(t, J = 6.0 Hz, 8H, N-$CH_3$) ; 3.15(t, J = 5.7 Hz, 4H, $CH_2$-N) ; 4.40(t, J = 5.7 Hz, 4H, O-$CH_2$) ; 6.70(d, J = 4.9 Hz, 2H Ar) ; 8.90(d, J = 4.9 Hz, 2H, Ar) ; 9.10(s, 1H, Ar) |
| **1g** | $-(CH_2)_2-N$  | 24 | 35 | 180-182 | 1.50(m, 4H, $CH_2$) ; 1.65(m, 8H,$CH_2$) ; 2.65(t, J = 5.5 Hz, 8H, N-$CH_2$) ; 3.00(t, J = 5.8 Hz 4H , $CH_2$-N) ; 3.35(s, 3H $CH_3$) ; 4.40(t, J = 5.8 Hz, 4H, O-$CH_2$) ; 6.70(d, J = 5.0 Hz, 2H, Ar) ; 8.90(d, J = 4.9 Hz 2H, Ar) ; 9.05(s, 1H, Ar) |
| **1h** | $-(CH_2)_2-N$  | 36 | 20 | 166-168 | 2.70(t, J = 4.6 Hz 8H, N-$CH_2$) ; 3.05(t, J = 5.6 Hz, 4H, $CH_2$-N) ; 3.35(s, 3H, $CH_3$) ; 3.75(t, J = 4.6 Hz, 8H, $CH_2$-O) ; 4.40(t, J = 5.6 Hz, 4H, O-$CH_2$) ; 6.70(d, J = 5.0 Hz, 2H, Ar) ; 8.90(d, J = 5.0 Hz, 2H, Ar) ; 9.00(s, 1H, Ar) |

* For compounds as free bases, except for **1c** (dihydrochloride)

** Analyses agree within +/- 0.4% of the theoretical values

*** Except for **1c** ($D_2O$/TMS)

Biological data are given in Table 2.

Table 2 : Cytotoxicity against L1210 and intercalation into DNA of dialkoxy pyridoquinolines 1.

| Compound | Cytotoxicity | Intercalation | | |
|---|---|---|---|---|
| | ($IC_{50}$, μM) | Bathochromic shift (nm) | Binding affinity ($x10^6$) | Number of sites |
| 1a | 47 | - | - | - |
| 1b | 33.7 | - | - | - |
| 1c | 3.7 | 2 | 6.89 | 0.45 |
| 1d | 1.7 | 2 | 3.44 | 0.33 |
| 1e | 0.45 | 2 | 6.18 | 0.44 |
| 1f | 2.5 | 2 | 2.14 | 0.17 |
| 1g | 3.5 | 2 | 6.24 | 0.25 |
| 1h | 30 | 6 | 2.67 | 0.24 |

As previously noted in the acridine series [4,5], intercalation occurs only when simultaneously exist a ring system as planar as possible and a protonatable nitrogen. However, there are no correlations between DNA affinity the cytotoxic potency.

Yet, alkyl substituents do not favour cytotoxicity whilst the best activity is observed with alkylaminoalkyl side chains, except in case of compound 1h. Undoubtly, the most potent compound is 1e. However, activity is about twenty times less than that of adriamycin ($IC_{50}$ = 0.025 μM).

Added to this, the antitumor activity "in vivo" is only moderate while toxicity rapidly increases as shown in Table 3.

Table 3 : Antitumor effects of compound 1e in P388 leukemia bearing mice.

| Dose (mg/kg) | Body weight change (%) | T/C (%) |
|---|---|---|
| 50 | 19 | 120 |
| 100 | 16 | 136 |
| 200 | 23 | 132 |
| 400 | - | 18 |

Hence, despite compounds prepared have no strong future as anticancer agents, the pyridoquinoline-dione moiety could be considered with interest in the concerned field.

**EXPERIMENTAL**

**1- Chemistry**

Melting points were determined in open capillary tubes on a Buchi-Tottoli apparatus and are given uncorrected. NMR spectra were recorded at 20.00 ± 0.1°C, using a Bruker AM 200 spectrometer. Microanalyses were carried out using a Technicon CHN autoanalyzer.

**General procedure**

A mixture of 10-methyl pyrido[3,2-g]quinoline-4,6-dione (5 mmol), alkyl halide (12.5 mmol) and toluene (20 ml), 50% aqueous potassium hydroxide (10 ml) and tetrabutylammonium bromide (2.5 mmol), was refluxed under stirring for 24 - 48 hrs. The organic layer was separated, washed with water and dried over sodium sulfate. After evaporation of the solvent, the solid obtained was dissolved in methanol (10 ml) and precipitated with ether. Recrystallization of the resulting solid in absolute ethanol gave the desired compounds 1.

**2 - Binding to DNA**

Intercalation of ligands into calf thymus DNA was spectrophotometrically measured. Spectrophotometric measurements were carried out in 0.018 mol/l NaCl - 0.03 mol/l Tris Cl Buffer (pH = 7.0), using a Pye-Unicam SP 8000 spectrophotometer fitted with a Pye-Unicam SP 876 series 2 temperature programme controller. Conditions were those previously described [4]. Binding constants were calculated using "Enzfitter", a non linear regression analysis programme from Elsevier Biosoft.

**3 - Cell toxicity**

Attention was focused on the cytoxicity against L1210 leukemia cells using procedures from Alley et al. [7] et Skehan et al. [8]. Results were compared to those obtained with adryamicin in the same experimental conditions.

**4 - "In vivo" study**

The selected drug 1e was intraperitoneally (i.p.) delivered to mice inoculated i.p. with P 388 leukemia cells ($10^{16}$ per mouse). The antitumor effect was evaluated by the percent T/C value ; the T/C being the ratio of the medium survival time (mst) in days of treated mice on the mst of control. In addition, variations in body weight which reflect the general toxic effect of a drug, were appreciated. Results are given in percent, still comparing treated animals versus controls.

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