SYNTHESIS OF A BENZODIOXINIC ANALOGUE OF ELLIPTICINE AND EVALUATION OF ITS ANTITUMOR ACTIVITY

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Abstract : A benzodioxinic analogue of Ellipticine was synthesized, using two strategies both involving a Diels Alder cycloaddition of furobenzodioxin. Antitumor activity was evaluated *in vitro* and *in vivo* on B 16 melanoma and P 388 Leukemia..

Introduction

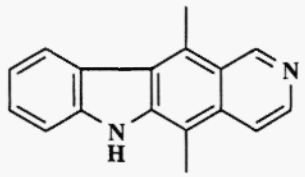
Ellipticine 1 (5,11-dimethyl-6H-pyrido [4,3-b] carbazole) is a naturally-occuring alkaloid which is known for its broad spectrum of antineoplastic activity (1). A number of structure-activity studies have already been made to determine the essential structural requirements associated with its antitumoral activity.

In particular, it was shown that the replacement of the carbazole nitrogen atom by either sulfur or oxygen resulted in loss of activity (2) and that substituting either benzoxathiine or benzothiazine substructures for the indolo heterocyclic nucleus also resulted in loss of activity (2). However, in these two cases the biological studies were performed with demethylated analogues of ellipticine and it had been previously shown that the presence of the 5 and 11 methyl groups was critical for activity (3,4).

Through other studies, W. A. Denny identified dibenzo [1,4] dioxin as a novel DNA-intercalating chromophore with *in vivo* antitumor activity (5).

Moreover we showed that tetracyclic dioxinocoumarines exhibited promising biological activities and in particular inhibited the growth of HeLa cells whether UVA radiations were present or not (6).

With the aim to investigate its biological properties as a potential antitumor agent we therefore synthesized a tetracyclic hybrid molecule 2 that includes a benzodioxinic substructure and the isoquinoline moiety of ellipticine.



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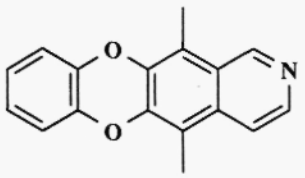

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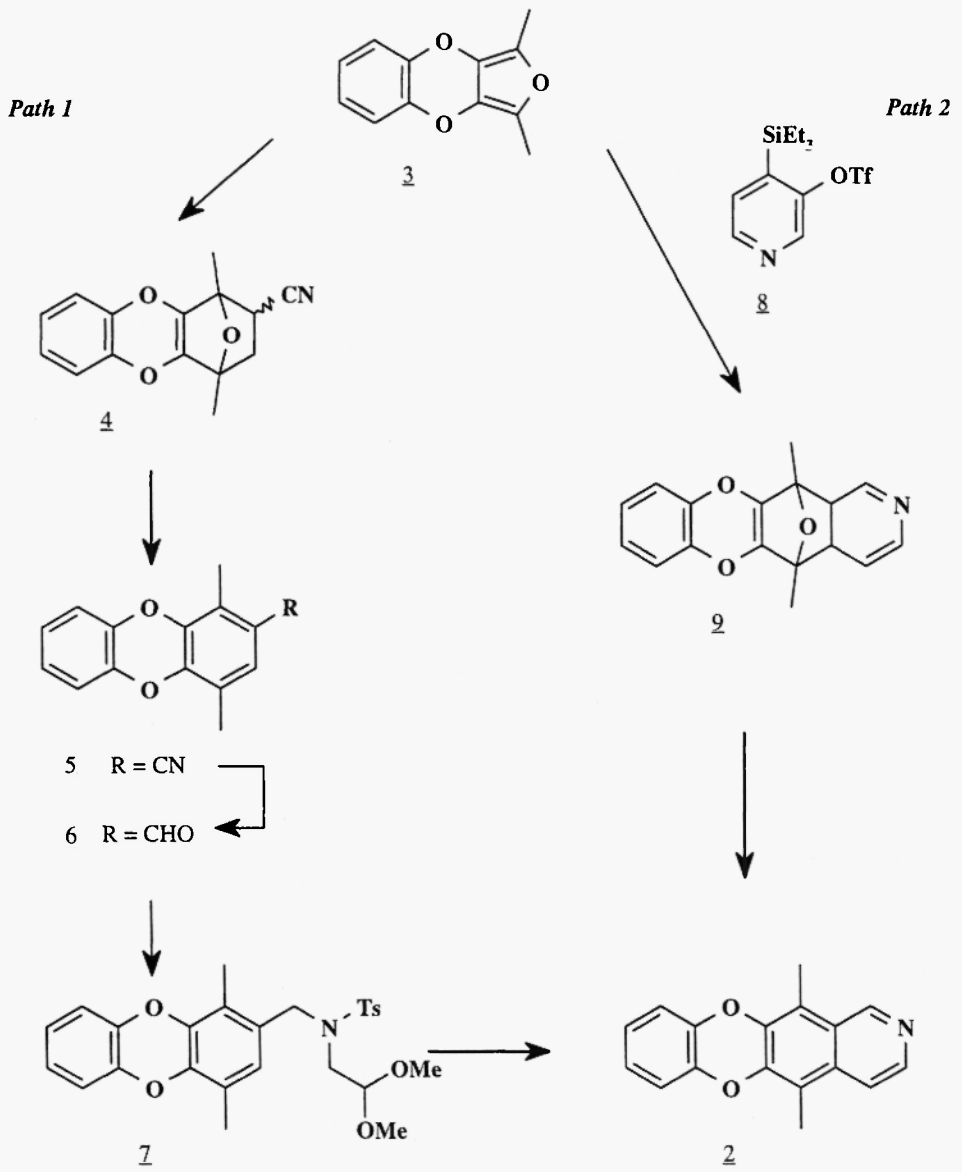
Results and Discussion

Chemistry

Compound 2 was synthesized following two distinct ways both involving a Diels-Alder cycloaddition with the previously described furobenzodioxin 3 as a key intermediate (7).

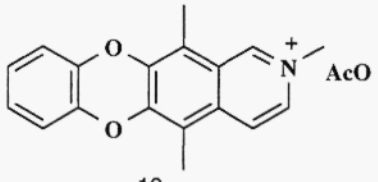


3 4 8 9 7 2

Access to target derivative 2 was first envisaged *via* the formyldibenzodioxin 6 and the subsequent elaboration of the isoquinoline ring using classical Pomeranz-Fritsch methodology (*path 1*). Furobenzodioxin 3 appeared to be very reactive in Diels-Alder reactions and reacted with acrylonitrile to yield a mixture of *endo* and *exo* adducts 4 which were immediately converted into nitrile 5 after treatment with potassium tert-butoxide (95%). The reduction of 5 with diisobutylaluminium hydride provided the desired aldehyde 6 in 96% yield. Treatment of 6 with aminoacetaldehyde dimethylacetal, reduction of the resulting imine, then tosylation of the amine led to 7 in high yield (91%). Compound 7 was converted into the required tetracyclic compound 2 by cyclisation under acidic conditions (62%).

Although the overall yield was quite satisfactory (50% from 3), we turned our attention towards an alternative and more direct approach involving a Diels-Alder reaction between 3 and highly reactive 3,4-didehydropyridine (*path 2*). The hetaryne was obtained according to Snieckus's procedure (8) by treatment of triflate 8 with tetrabutylamonium fluoride, and reacted with 3 to lead to adduct 9 in 65% yield (9). Deoxygenation of 9 with lithium aluminium hydride in the presence of titanium (IV) chloride (10) provided 2 in 56% yield.

Finally the tetracyclic derivative was treated with methyl iodide and the resulting iodide salt converted by ion-exchange chromatography into acetate 10, for biological study.



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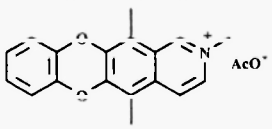
Pharmacology

Cytotoxicity tests were performed *in vitro* by colony forming assay on B16 mouse melanoma cells. 50 % inhibition doses (IC_{50}) were measured as concentrations giving a 50% cloning efficiency (11). *In vivo* tests were performed on C57B16 male mice inoculated sc with B16 melanoma cells and ip with P388 leukemia cells. For each dose the effect was expressed as an oncostatic index I = T/C×100 where T was the median survival time of the group treated and C that of the control group (12). Animals were treated at days 1, 5 and 9 following inoculation with doses corresponding to 20% of the lethal dose 50% (LD_{50}) (2 mg/kg). The results are given in table 1:

Table 1 : *In vitro* cytotoxicity tests performed on B 16 melanoma cells and *in vivo* studies on B16 melanoma and P388 leukemia. For each *in vitro* tests, the value is the average of 5 experiments ± SD experiments. For the *in vivo* tests, the survival durations of batches of 10 tumour-bearing mice were observed for 60 days.

	In vitro test, IC_{50} (μM)	*In vivo* test on B16 I (%)	*In vivo* test on P388 I (%)
	15.6 ± 3.9	100	125

These results show that compound 10 exhibits cytotoxicity *in vitro* against B16 melanoma cells. But these results are not confirmed by the *in vivo* tests although our compound exhibits a slight activity against P388 leukemia. This discrepancy between *in vivo* and *in vitro* tests could be due to a metabolic process inactivating these drugs. Studies are now in progress to determine the metabolic pathway of these molecules that could explain the inactivation observed *in vivo*. Other benzodioxinic analogues of ellipticine will be synthesized for the mechanism of action to be understood.

Experimental

Melting points were determined in open capillary tubes on a Büchi apparatus and were uncorrected. ^{1}H-NMR spectra were recorded on a Bruker AM-300 WB instrument at 300 MHz. ^{13}C-NMR spectra were recorded on a Varian-Gemini-200 instrument at 50.4 MHz. Mass spectra were recorded with a Ribermag R10-10 mass spectrometer. Infrared spectra were recorded on a Perkin Elmer 297 apparatus. All the reactions were carried out under an atmosphere of dry argon. Analytical thin-layer chromatography was performed on precoated silica gel 60 F-254 plates (0.2 mm thick). The reaction mixtures were chromatographed on silica gel columns (Merck 70-230 mesh).

2-cyano-1,4-dimethyl-1,4-epoxy-1,2,3,4-tetrahydrodibenzo [*b,e*] 1,4-dioxine) 4

A mixture of diene 3 (400 mg, 2.1 mmol) and acrylonitrile (182 mg, 3.15 mmol) was heated at 50°C in a sealed tube during two hours. A mixture of isomers endo and exo was obtained and purified by column chromatography (petroleum ether/ethyl acetate, 9/1). Yield 95 %.

2-cyano-1,4-dimethyldibenzo [*b,e*] 1,4-dioxine 5

A solution of 4 (250 mg, 0.98 mmol) and potassium *tert*-butoxide (155 mg, 1.27 mmol) in tetrahydrofuran (10 ml) was stirred at room temperature for five minutes hydrolysed with hydrochloric acid (0.1 M in water), the aqueous phase was extracted with ethyl acetate. The organic phase was dried with magnesium sulfate. The solvent was evaporated and the residue purified by column chromatography (petroleum ether/ethyl acetate, 8/2). Yield 91 %, mp = 144°C, IR (KBr): ν cm^{-1} 2210 (C≡N), 1590 (C=C), 1260 (C-O-C), RMN ^{1}H ($CDCl_3$): δ ppm 2.22 (s, 3H, $\mathbf{CH_3}$-C_4), 2.38 (s, 3H, $\mathbf{CH_3}$-C_1), 6.91 (m, 4H, H_6,H_7,H_8,H_9), 7.03 (s, 1H, H_3), RMN ^{13}C ($CDCl_3$) δ ppm 13.4, 14.8, 116.3, 117.7, 123.0, 124.2, 124.4, 127.0, 129.0, 140.3, 141.1, 141.3, 143.9, 170.3.

1,4-dimethyl-2-formyldibenzo [*b,e*] 1,4-dioxine 6

A solution of diisobutylaluminium hydride in toluene (6.3 ml, 6.3 mmol) was added dropwise to a solution of 5 (1 g, 3.9 mmol) in dry toluene (120 ml). After a 15 minutes stirring at room temperature the reaction mixture was cautiously hydrolysed at 0°C with an aqueous 2M solution of hydrochloric acid. The aqueous phase was extracted with ethyl acetate. The combined organic phases were dried, then evaporated. The residue was purified by column chromatography (petroleum ether/ethyl acetate, 8/2) and 6 was obtained as a pale yellow solid. Yield 92 %, mp = 255°C, IR (KBr) : ν cm^{-1} 2850 (C-H), 1680 (C=O), 1590 (C=C), 1250 (C-O-C), RMN ^{1}H ($CDCl_3$) : δ ppm 2.25 (s, 3H, $\mathbf{CH_3}$-C_4), 2.51 (s, 3H, $\mathbf{CH_3}$-C_1), 6.9 (m, 4H, H_6,H_7,H_8,H_9), 7.25 (s, 1H, H_3), 10.05 (s, 1H, **CHO**), RMN ^{13}C ($CDCl_3$) δ ppm 9.9, 14.8, 116.2, 126.0, 123.8, 124.2, 126.0, 129.4, 140.0, 141.1, 141.4, 191.0.

1,4-dimethyl-2-(N-tosyl-2,2-dimethoxyethylamino)dibenzo [*b.e*] 1,4-dioxine 7

A solution of 6 (860 mg, 3.58 mmol) and aminoacetaldehyde dimethylacetal (0.6 ml, 5.37 mmol) in dry toluene (55 ml) was refluxed for 6 hours in the presence of molecular sieves (5 Å). The solvent was filtered and evaporated then the crude imine was dissolved in 150 ml of ethanol and stirred for one night at room temperature under an hydrogen atmosphere with platinum oxide as a catalyst. The reaction mixture was then filtered and the solvent was evaporated.

The crude amine was dissolved in dichloromethane (90 ml) and the solution was cooled to 0°C. A solution of triethylamine (1.5 ml, 11.2 mmol) and tosyl chloride (1.07 g, 5.6 mmol) in dichloromethane (55 ml) was then added dropwise and left for 14 hours at room temperature then a saturated solution of sodium hydrogenocarbonate was added. The organic phase was separated and the aqueous phase extracted with dichloromethane. The combined organic phases were dried with magnesium sulfate. The solvent was evaporated and compound 7 obtained as a brown solid. Yield 97 %, mp = 92°C, IR (KBr): ν cm^{-1} 1600 (C=C), 1320 (SO_2), 1260 (C-O-C), 1150 (SO_2), RMN ^1H ($CDCl_3$): δ ppm 2.09 (s, 3H, CH_3-C_1), 2.14 (s, 3H, CH_3-C_4), 2.42 (s, 3H, CH_3-$C_{4''}$), 3.18 (d, 2H, $H_{3'}$, J=5,1 Hz), 3.25 (s, 6H, CH_3-O-), 4.31 (t, 1H, $H_{4'}$, J=5,1 Hz), 4.32 (s, 2H, $H_{1'}$), 6.46 (s, 1H, H_3), 6.87 (m, 4H, H_6,H_7,H_8,H_9), 7.31 (d, 2H, $H_{3''}$,$H_{5''}$, J=8,1 Hz), 7.71 (d, 2H, $H_{2''}$,$H_{6''}$), RMN ^{13}C ($CDCl_3$) δ ppm 10.4, 14.8, 21.4, 49.0, 50.7, 54.5, 103.7, 116.0, 116.1, 123.5, 125.5, 127.2, 129.5, 136.6, 140.0, 141.9, 143.3.

5,12-epoxy-5,12-dimethyl-5,12-dihydro-1,4-benzodioxino [2,3-*g*] isoquinoline 9

A mixture of 2 ml of tetrabutylamonium fluoride (1 M in tetrahydrofuran) and acetonitrile (6 ml) was stirred for one hour at room temperature in the presence of molecular sieves (4 Å).A solution of 3 (50 mg, 0.25 mmol) and triflate 8 (507 mg, 1.5 mmol) in acetonitrile (6 ml) was then slowly added in 15 minutes at 0°C. Stirring was continued for one hour at this temperature, then the reaction mixture was filtered and diluted with ethyl acetate (30 ml). After being washed with a saturated solution of sodium chloride, the organic phase was dried with magnesium sulfate, filtered and evaporated Compound 9 was isolated after column chromatography (petroleum ether/ethyl acetate, 8/2) as a pale yellow solid. Yield 55 %, mp = 141°C, IR(KBr) : ν cm^{-1} 1715 (C=C), 1220 (C-O-C), RMN ^1H ($CDCl_3$, 300 MHz): δ ppm 1.80 (s, 3H, CH_3-C_5), 1.86 (s, 3H, CH_3-C_{12}), 6.78 (m, 2H, H_7,H_{10}), 6.81 (m, 2H, H_8,H_9), 7.17 (d, 1H, H_4, J=4.6 Hz), 8.42 (d, 1H, H_3, J=4.6 Hz), 8.44 (s, 1H, H_1).

5,12-dimethyl-1,4-benzodioxino [2,3-*g*] isoquinoline 2

Path 1 : A solution of 7 (2 g, 4.2 mmol) in dioxane (140 ml) and concentrated hydrochloric acid (40 ml) was heated at 70°C and stirred for 4 hours. The dioxane was then removed and the aqueous phase extracted with ethyl acetate. A solution of sodium hydroxide (20 % in water) was added and the aqueous phase extracted with ethyl acetate. The organic solvent was dried over magnesium sulfate, then evaporated. Derivative 2 was obtained after purification by column chromatography (petroleum ether/ethyl acetate /triethylamine 6/4/1) as a white solid. Yield 62 %.

Path 2 : Lithium aluminium hydride (17 mg, 0.44 mmol) was added to a solution of titanium (IV) chloride (0.12 ml, 1 mmol) and triethylamine (0.21 ml, 1.5 mmol) in tetrahydrofuran (8 ml). The resulting mixture was refluxed for 10 minutes then cooled to room temperature. A solution of 9 (56 mg, 0.2 mmol) in tetrahydrofuran (5 ml) was added and the reaction mixture was refluxed for 24 hours. After cooling and hydrolysis with a concentrated solution of ammonium hydroxide, the aqueous phase was extracted with ethyl acetate. The solvent was dried over magnesium sulfate, then removed and compound 2 purified by column chromatography (petroleum ether/ethyl acetate /triethylamine 6/4/1). Yield 55 %, mp = 144°C, IR (KBr): ν cm^{-1} 1620 (C=C), 1190 (C-O-C), RMN ^1H ($CDCl_3$): δ ppm 2.49 (s, 3H, CH_3-C_5), 2.41 (s, 3H, CH_3-C_{12}), 6.98 (m, 4H, H_7,H_8,H_9,H_{10}), 7.58 (d, 1H, H_4, J=5.9 Hz), 8.42 (d, 1H, H_3, J=5.9Hz), 9.22 (s, 1H, H_1), RMN ^{13}C ($CDCl_3$) δ ppm 9.4, 9.7, 116.7, 116.9, 124.4, 124.7, 124.9, 125.0, 133.1, 138.9, 140.7, 140.8, 141.6, 142.6, 148.5.

Acknowledgements : We are most grateful to the *Ligue Nationale contre le Cancer* (France) for its grant.

References and notes :

(1). Gribble, G. W., in *The Alkaloids*, Brossi, A., Ed., Academic Press, 1990, Vol 39, pp. 239-352.

(2). Sengupta, D.; Anand, N.; *Indian J. Chem.*, Sect. B, **1986**, *25 B*, 72.

(3). Sainsbury, M., *Synthesis*, **1977**, 437.

(4). Archer, S., Ross, B. S., Pica-Mattoccia, L., Cioli, D., *J. Med. Chem.*, **1987**, *30*, 1204.

(5). Palmer, B. D.;Rewcastle, G. W.; Atwell, G. J.; Baguley, B. C.; Denny, W. A., *J. Med. Chem*, **1988**, *31*, 707.

(6). (a) Guillaumet, G.; Hretani, M.; Coudert, G.; Averbeck, D.; Averbeck, S.; *Eur. J. Med. Chem.*, **1990**, *25*, 45. (b) Csik, G.; Besson, T.; Coudert, G.; Guillaumet, G.; Nocentini, S., *J. Photochem. Photobiol. B : Biol.* **1993**, *19*, 119. (c) Csik, G.; Ronto, G.; Nocentini, S.; Averbeck, S.; Averbeck, D.; Besson, T.; Coudert, G.; Guillaumet, G., *J. Photochem. Photobiol. B : Biol.*, **1994**, *24*, 129.

(7). Ruiz, N.; Buon, C.; Pujol, M. D.; Guillaumet, G.; Coudert, G., *Synth. Commun.* **1996**, *26*, 2057.

(8). Tsukazaki, M.; Snieckus, V., *Heterocycles*, **1992**, *33*, 533.

(9). It was necessary to use 6 equivalents of 8 to obtain adduct 9 as a single compound in 65% yield.

(10). Wong, H. N. C.; Xue Long Hou, *Synthesis*, **1985**, 1111.

(11). Freshney, R. I., *Culture in animal cells, a manual of basic techniques*, Alan R. Liss ed., New York, **1985**.

(12). Bourrut, C.; Chenu, E.; Godeneche, D.; Madelmont, J. C.; Maral, R.; Mathe, G.; Meyniel, G.; *Br. J. Pharmac.*, **1986**, *89*, 539.

Received on July 31, 1997