A Photochemical Approach to Pyridopyrroloquinoline Derivatives as New Potential Anticancer Agents

Pierre-Jean Aragon,[a,b] Ange-Désiré Yapi,[a] Frédéric Pinguet,[b] Jean-Michel Chezal,[c] Jean-Claude Teulade,[c] Jean-Pierre Chapat,[a] and Yves Blache*,[d]

[a] *Laboratoire de Chimie Organique Pharmaceutique, E.A. 2414, Faculté de Pharmacie; 15 avenue Charles Flahault, 34060 Montpellier, France:* [b] *Laboratoire d'Oncopharmacologie, Centre Régional de Lutte Contre le Cancer; Val d'Aurelle, 34298 Montpellier cedex 05, France:* [c] *Laboratoire de Chimie Organique Pharmaceutique, Faculté de Pharmacie, UMR INSERM 484; 28 Place Henry Dunant, B.P. 38, 63001 Clermont-Ferrand, France: and* [d] *Laboratoire de chimie organique et thérapeutique, EA 3660, Unité de Molécules d'Intérêt Biologique, Faculté de Pharmacie; 7 boulevard Jeanne d'Arc, BP 89700, 21079 Dijon, France.* Received November 25, 2003; accepted February 26, 2004

Indoloquinoline alkaloid cryptolepine and pyridocarbazole alkaloid ellipticine are of great interest because *in vitro* and *in vivo* studies revealed their good cytotoxic properties. In order to obtain some biologically active analogs of these compounds, we developped a synthesis based on the photocyclisation of tertiary *N*-methylated enaminones derived from cyclopentane-1,3-dione and 3 or 6-aminoquinoline. The angular cyclised compounds thus obtained were submitted to Beckmann rearrangement, preceded by the formation of a Z oxime. Finally, the δ-lactame ring was oxidized using 10% palladium/carbon in diphenylether and pyridopyrroloquinolines were obtained. These compounds and the intermediate lactams and cyclopentanopyrroloquinolines were tested *in vitro* on K 562 cells and A 2780 doxorubicine sensitive and resistant cells. All compounds were less effective than doxorubicine in sensitive cells but their activity wasn't decreased by MDR resistance.

Key words photochemistry; pyridopyrroloquinoline; cytotoxicity; enaminone; Beckmann

The pyridocarbazole alkaloid ellipticine[1] **1** is well known for its high cytotoxicity against several cancer cell lines due to its intercalating properties and its ability to inhibit DNA religation by topoisomerase II.[2,3] A number of angular analogs have been prepared in order to obtain more active compounds. For example, one of the most promising products appeared to be intoplicine **2**, synthesised firstly by Nguyen *et al.*,[4] which acts both as an intercalating agent and as a topoisomerase I and II inhibitor.[5,6]

So, considering the cytototoxic properties of angular nitrogenous heterocycles, other groups have elaborated several angular tetracyclic compounds derived from two or three nitrogenous heterocycles. Dalla Via *et al.*[7] prepared indolonaphtyridines **3** carrying a dialkylaminoalkyl side chain and different substituents. Linear flow dichroism studies demonstrated these compounds were able to intercalate into DNA and *in vitro* cytotoxicity studies showed IC_{50} on HL-60 cells varying between 0.5 and 1.6 μM. Furthermore, Da Settimo *et al.*[8] prepared several derivatives of purinoquinazoline **4**, pyridopyrimidopurine and pyridopyrimidobenzimidazole, all of them carrying a dialkylaminoalkyl side chain. Only purinoquinazolines could bind strongly to DNA and therefore could induce DNA double-strand breaks *via* inhibition of DNA religation by topoisomerase II. These compounds showed IC_{50} on HL-60 cells varying between 0.072 and 0.47 μM. Chart 1 represents some of the structures mentionned above.

Therefore, as a part of our studies related to the pharmacochemistry of angular polynitrogenous tetracycles,[9,10] we initiated a program in order to examine the synthesis of new pyridopyrroloquinolines and their antitumor activities against resistant cell lines (MDR phenotype +). Our synthetic methodology resides in the use of enaminones **5** derived from quinolines and cyclopentane-1,3-dione. Key steps of the synthesis are the photocyclisation of such enaminones followed by Beckmann rearrangement to afford hydroxypyridine ring from 2-cyclopent-1-one ring, as shown in Chart 2. In this context, we have previously described the synthesis of indoloquinolines and pyridocarbazoles by photocyclisation of enaminones derived from 3 or 6-aminoquinoline and cyclohexane-1,3-dione.[11,12] This present work will allow us to study the photoreactivity of new enaminones and to elaborate potentially cytotoxic compounds.

Results and Discussion

Chemistry Firstly, we studied the reactivity of secondary halogenated enaminones **12** and **13**. As shown in Chart 3, these compounds were obtained in two steps by con-

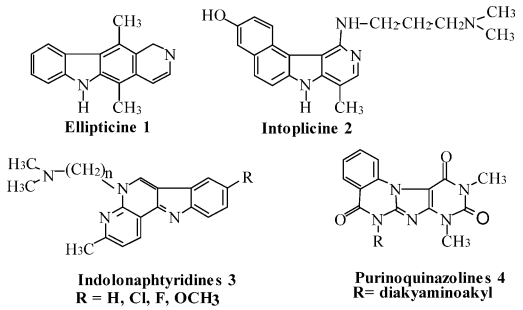

Chart 1

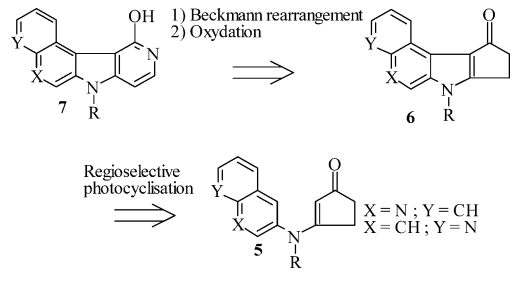

Chart 2

* To whom correspondence should be addressed. e-mail: yves.blache@u-bourgogne.fr

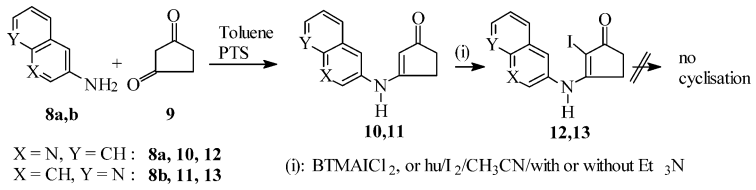

Chart 3

Table 1. Irradiation of Enaminones **12** and **13**

Enaminone	Irradiation conditions	Starting material	Dehalogenated product
12	CH_3CN, Et_3N, 4 h 00	**12**; 0%	**10**; 80%
	CH_3CN, 4 h 00	**12**; 80%	**10**; 20%
13	CH_3CN, Et_3N, 4 h 00	**13**; 0%	**11**; 95%
	CH_3CN, 4 h 00	**13**; 100%	**11**; 0%

Table 2. Irradiation of Enaminones **10** and **11**

Enaminone	Irradiation conditions	Starting material	Halogenated product
10	CH_3CN, I_2, Et_3N, 4 h 00	**10**; 40%	**12**; 40%
	CH_3CN, I_2, 4 h 00	**10**; 40%	**12**; 60%
11	CH_3CN, Et_3N, I_2, 4 h 00	**11**; 20%	**13**; 80%
	CH_3CN, I_2, 4 h 00	**11**; 0%	**13**; 100%

densation of 3 or 6-aminoquinoline (**8a**, **b**) with cyclopentane-1,3-dione in toluene with paratoluenesulfonic acid, followed by α-iodination of **10** and **11** using $BTMAICl_2$, according to the procedures previously described.[11,13)]

Irradiation of enaminones **12** and **13** in acetonitrile with or without triethylamine gave a mixture of starting product and dehalogenated product, as shown in Table 1. Contrary to α-iodinated enaminones derived from 3 or 6-aminoquinoline and cyclohexane-1,3-dione, no cyclisation occurred.[11)]

Another attempt at photocyclisation was conducted according to the one-pot synthesis of pyridocarbazole and indoloquinoline previously described.[11)] Compounds **10** and **11** were irradiated in acetonitrile +/− triethylamine in presence of iodine (2 eq) for 4 h. But only iodinated products **12** and **13** were obtained without any cyclised products, as shown in Table 2. So, when iodinated enaminones derived from aminoquinolines and cyclopentane-1,3-dione undergo irradiation, the competition between dehalogenation and cyclisation[13)] is clearly in favour of dehalogenation. As expected, this dehalogenation is enhanced by the presence of triethylamine, particularly in the case of 6-aminoquinoline derivatives. Chart 3 recapitulates these attempts.

Since secondary halogenated enaminones didn't undergo photocyclisation, the reactivity of tertiary enaminones **14** and **15** was investigated (Chart 4). Previous works have been conducted by Gardette *et al.*[14)] who cyclised enaminones derived from *N*-methylaniline and cyclopentan-1,3-dione. These compounds were prepared by *N*-methylation of enaminones **10** and **11** using methyl iodide in toluene in presence of sodium hydride. Irradiation of such compounds under different conditions, as shown in Table 3, produced the best yield for cyclised compounds **16** and **17** when a mixture toluene/methanol (95/5) in a quartz reactor was being used, proving thus that photocyclisation requires much energy. The

Table 3. Irradiation of Enaminones **14** and **15**

Enaminone	Irradiation conditions	Cyclised product
14	Pyrex, MeOH, 4 h 00	**16**; 20%
	Pyrex, toluene/MeOH (50/50), 4 h 00	**16**; 30%
	Pyrex, toluene/MeOH (95/5), 4 h 00	**16**; 45%
	Quartz, toluene/MeOH (95/5), 4 h 00	**16**; 50%
15	Pyrex, MeOH, 4 h 00	**17**; 5%
	Pyrex, toluene/MeOH (50/50), 4 h 00	**17**; 5%
	Pyrex, toluene/MeOH (95/5), 4 h 00	**17**; 15%
	Quartz, toluene/MeOH (95/5), 4 h 00	**17**; 25%

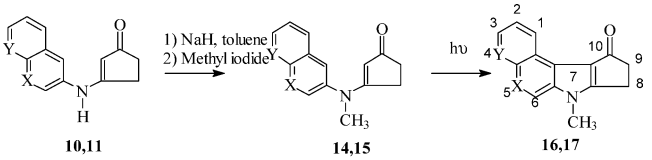

Chart 4

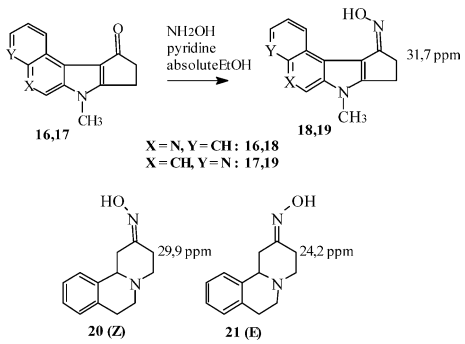

Chart 5

position of the intracyclic nitrogen atom seems to influence the reactivity of these compounds because photocyclisation yield is two fold smaller in the case of compound **15**, derived from 6-aminoquinoline.

This photocyclisation appears to be regioselective, since we obtain only angular derivatives. The angular structure of these compounds is proven by the multiplet corresponding to H1, and whose shift in ^{1}H-NMR spectrum appears at 8.77 ppm for compound **16** and 9.17 ppm for compound **17**, due to the deshielding effect of the carbonyl group. Besides, compound **16** shows a characteristic singlet corresponding to H6 at 8.76 ppm and compound **17** shows a characteristic AB system corresponding to H5 and H6 at 7.57 ppm.

Thus, the cyclopentenone ring has been modified in three steps including Beckmann rearrrangement in order both to introduce a third nitrogen atom and to obtain some totally aromatised compounds which can theoretically better inter-

calate into DNA.

First of all, the compounds **16** and **17** were treated by hydroxylamine hydrochloride, following the procedure described by Sekar *et al.*[15] and Z oximes **18** and **19** were obtained. Configuration of C=N bond was determined by considering the ^{13}C-NMR shift of the secondary carbon adjacent to the oxime function in our structures and in close compounds **20** and **21** synthetised by Scheiber *et al.*,[16] as shown in Chart 5.

The second step of this ring modification consisted in regioselective Beckmann rearrangement[17] using polyphosphoric acid PPA, which gave lactams **22** (30%) and **23** (60%). The structure of the lactams was deduced from the configuration of the oximes, considering the mechanism of Beckmann transposition, and was confirmed by considering the ^{13}C-NMR shift of the secondary carbon adjacent to the amide function in our structures and in close compounds **24** and **25** synthetised by Scheiber *et al.*,[16] as shown in Chart 6.

In order to obtain compounds **22** and **23** with better yields, compounds **16** and **17** were submitted to Schmidt transposition[17] using sodium azide in refluxing sulfuric acid. But after 3 h, the starting material was recovered unchanged.

Finally, compounds **22** and **23** were treated by palladium/carbon 10% in diphenylether[18] and underwent C_8–C_9 bond oxydation to give the pyridopyrroloquinolines **26** and **27** with 30% and 32% yield respectively, as shown in Chart 7. This oxydation was expected to be more successful than previous attempts to oxydize cyclohexenone ring, since lactime tautomer in δ-lactam ring is probably more easely formed than enol tautomer in cyclohexenone ring.

Biological Results In continuation of our previous works[9,10] concerning the antiproliferative activity of tetracyclic nitrogenous heterocycles, and more precisely, their activity against MDR$^+$ cancer cells lines, the cytotoxicity of the compounds **16**, **17**, **22**, **23**, **26** and **27** was evaluated by a cell growth inhibition assay against two human cell lines: K 562 (leukemia), and A 2780 (ovarian cancer) doxorubicine-sensitive and resistant (MDR$^+$) and was compared to the cytotoxicity of doxorubicine. The resistant subline A 2780 R was established by the continuous exposure of cells to gradually increasing concentrations of doxorubicine. The resistant subline K 562 R wasn't tested because of the poor activity of our products against K 562 S cells. IC_{50} (concentration inhibiting 50% of the cell proliferation) expressed in mol/l and resistance factor (IC_{50} on A2780 resistant cells/IC_{50} on A2780 sensitive cells) of each compound are recapitulated in Table 4. IC_{50} of compound **22** couldn't be determined exactly because of its poor solubility in the culture medium.

All the compounds were less effective than doxorubicine in all sensitive cells, whereas compounds **17**, **23**, **26** and **27** were as active as doxorubicine on A 2780 resistant cells with IC_{50} varying between 5 and 9.8×10^{-6} mol/l. The resistance factor of all those compounds (except **22**, not determined) varies between 1.1 and 6.1 (doxorubicine: 121), which indicates that these compounds are not concerned by the multidrug resistance phenomenon.

Among these compounds, the less active ones are those which possess δ-lactam ring. This can be due either to the poor solubility of these structures in the culture medium or to the structure of the compound itself. Besides, compounds derived from 6-aminoquinoline are globally more active than compounds derived from 3-aminoquinoline, proving the importance of the quinolinic nitrogen position in biological activity. But there's only a little difference between the compounds **16** and **17** carring a cyclopentenone ring and the compounds **26** and **27** carrying an hydroxypyridine ring.

Compounds **16**, **17**, **26** and **27**, which show the lowest IC_{50}, are good candidates for further *in vivo* studies.

Conclusion

In this paper, we have reported the synthesis of tetracyclic nitrogen heterocycles: pyrido pyrroloquinolines obtained from cyclopentanopyrroloquinolines *via* a Beckmann rearrangement. The photochemical step which gives cyclopentanopyrroloquinolines from enaminones occured with a total regioselectivity, since only angular compounds are obtained. The first *in vitro* studies reveal that the main interest of these

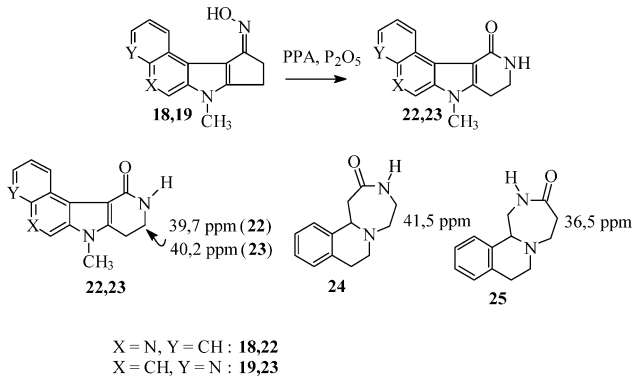

Chart 6

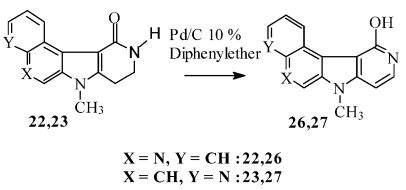

Chart 7

Table 4. IC_{50} and Resistance Factor of Tested Compounds and Doxorubicine

Compounds	K 562 cells (mol/l)	A 2780 cells sensitive (mol/l)	A 2780 cells resistant (mol/l)	Resistance factor
16	$4.9\times10^{-5}\pm1.1\times10^{-5}$	$3.3\times10^{-5}\pm2.5\times10^{-5}$	$8.8\times10^{-5}\pm5.4\times10^{-5}$	2.6
17	$5.8\times10^{-5}\pm1.2\times10^{-5}$	$2.7\times10^{-6}\pm1.5\times10^{-6}$	$6.3\times10^{-6}\pm5.6\times10^{-7}$	2.3
22	$>4.2\times10^{-4}$	$>4.2\times10^{-4}$	$>4.2\times10^{-4}$	n.d.
23	$7.7\times10^{-5}\pm1.9\times10^{-5}$	$1.6\times10^{-5}\pm7\times10^{-6}$	$9.8\times10^{-6}\pm9.4\times10^{-7}$	6.1
26	$5\times10^{-5}\pm1\times10^{-5}$	$4.5\times10^{-6}\pm1\times10^{-7}$	$5\times10^{-6}\pm6\times10^{-7}$	1.1
27	$1.1\times10^{-5}\pm8.3\times10^{-6}$	$5.7\times10^{-6}\pm7\times10^{-7}$	$8.5\times10^{-6}\pm3.8\times10^{-7}$	1.5
Doxorubicine	$1.6\times10^{-7}\pm3.3\times10^{-8}$	$2.4\times10^{-8}\pm4\times10^{-9}$	$2.9\times10^{-6}\pm4.9\times10^{-7}$	121

compounds resides in their activity toward resistant cells. Further studies on the pharmacomodulation of such compounds are now in progress and should lead to an interesting class of new potential anticancer agents with no multidrug resistance phenomena.

Experimental

IR spectra were recorded with a Perkin–Elmer 377 spectrophotometer. Absorption bands are expressed in centimeters (cm^{-1}) using polystyrene calibration and only noteworthy absorptions are listed. ^{1}H- and ^{13}C-NMR spectra were recorded on a Brüker AC 100 or EM 400 WB. Chemical shift data are reported in ppm downfield δ from TMS. Coupling constants J are given in Hz. s, d, dd, t, and m mean respectively singlet, doublet, double doublet, triplet, and multiplet. Mass spectrometry was done on a LKB 2091 instrument at 15 eV. Melting points were determined on a Buchi capillary melting point apparatus and are not corrected. Elemental analysis was performed by Microanalytical center, ENSCM, Montpellier.

Irradiations were conducted in a pyrex or a quartz well apparatus using a medium pressure mercury UV lamp (Heraeus TQ 150). Total volume of solvent(s) was about 350—400 ml. In order to remove oxygen, the reaction mixture was submitted to ultrasound waves for 10 min before irradiation. Besides, during the irradiation, the reaction mixture was flushed with a stream of nitrogen. Triethylamine (4 ml) or molecular iodine (500 mg, 1.97 mmol) could be added to the reaction mixture.

3-[3′-Quinolinylamino]cyclopent-2-en-1-one 10 A solution of 3-aminoquinoline (3 g, 21 mmol), 1.3 cyclohexanedione (2.2 g, 22 mmol) and paratoluenesulfonic acid (500 mg, 2.9 mmol) in 50 ml of anhydrous toluene was refluxed under nitrogen in a Dean–Stark apparatus for 6 h. After cooling, the solution was washed with water and extracted with dichloromethane. Organic layers were dried over sodium sulfate and evaporated *in vacuo*. The crude product was chromatographed on alumina gel using dichloromethane and a gradient of methanol as eluent to give **10** as a yellow powder: 40%, mp: 130—132 °C. ^{1}H-NMR ($CDCl_3$, 100 MHz) δ: 2.44 (2H, m, H5), 2.80 (2H, m, H4), 5.68 (1H, s, H2), 7.62 (3H, m, H5′, H6′ and H7′), 7.90 (2H, m, H4′ and H8′), 8.57 (1H, d, J=2.2 Hz, H2′); ^{13}C-NMR ($CDCl_3$, 100 MHz) δ: 29.19, 33.41, 102.86, 124.88, 127.78, 128.14, 128.47 (2C), 129.28, 134.21, 144.73, 145.39, 174.58, 208.34. *Anal.* Calcd for $C_{14}H_{12}N_2O$: C, 75.0; H, 5.36; N, 12.50. Found: C, 74.96; H, 5.32; N, 12.39.

3-[6′-Quinolinylamino]cyclopent-2-en-1-one 11 This compound was obtained in 25% yield according to the procedure described for compound **10**. White powder, mp: 134—136 °C. ^{1}H-NMR ($CDCl_3$, 100 MHz) δ: 2.39 (2H, m, H5), 2.76 (2H, m, H4), 5.67 (1H, s, H2), 7.31 (2H, m, H7′ and H3′), 7.49 (1H, s, H5′), 7.98 (2H, m, H4′ and H8′), 8.69 (1H, m, H2′); ^{13}C-NMR ($CDCl_3$, 100 MHz) δ: 29.3, 33.4, 102.8, 116.8, 122.2, 124.9, 129.2, 130.0, 136.5, 138.6, 145.1, 149.4, 174.2, 208.1. *Anal.* Calcd for $C_{14}H_{12}N_2O$: C, 75.0; H, 5.36; N, 12.50. Found: C, 75.04; H, 5.48; N, 12.42.

2-Iodo-3-[3′-quinolinylamino]cyclopent-2-en-1-one 12 A solution of enaminone **10** (250 mg, 1.1 mmol), benzyltrimethylammonium dichloroiodate $BTMAICl_2$ (390 mg, 1.1 mmol) and sodium bicarbonate (660 mg, 7.7 mmol) in 40 ml of anhydrous dichloromethane and 20 ml of anhydrous methanol was stirred under nitrogen at room temperature for 1 h. The solution was filtered to eliminate sodium bicarbonate and the solvents were evaporated *in vacuo*. The residue was dissolved in dichloromethane and washed with water. The organic layer was dried over sodium sulfate and evaporated *in vacuo*. The crude product was chromatographed on alumina gel using dichloromethane and a gradient of methanol as eluent to give **12** as a yellow powder: 80%, mp: 104—106 °C. ^{1}H-NMR ($CDCl_3$, 100 MHz) δ: 2.53 (2H, m, H5), 2.82 (2H, m, H4), 7.53 (1H, m, H7′), 7.67 (1H, m, H6′), 7.76 (1H, d, J=8.1 Hz, H5′), 7.99 (2H, m, H4′ and H8′), 8.70 (1H, d, J=2.5 Hz, H2′); ^{13}C-NMR ($CDCl_3$, 100 MHz) δ: 29.1, 32.6, 70.7, 128.3 (2C), 128.7 (2C), 129.1, 130.6, 130.7, 146.2, 147.8, 175.5, 200.6. *Anal.* Calcd for $C_{14}H_{11}IN_2O$: C, 48.0; H, 3.14; N, 8.0. Found: C, 47.88; H, 3.17; N, 7.96.

2-Iodo-3-[6′-quinolinylamino]cyclopent-2-en-1-one 13 This compound was obtained in 80% yield according to the procedure described for compound **12**. Yellow powder, mp: 110—112 °C. ^{1}H-NMR ($CDCl_3$, 100 MHz) δ: 2.62 (2H, m, H5), 2.96 (2H, m, H4), 7.46 (1H, m, H3′), 7.57 (1H, d, J=6.5 Hz, H7′), 7.65 (1H, s, H5′), 8.1 (1H, d, J=4.4 Hz, H4′), 8.16 (1H, d, J=8.2 Hz, H8′), 8.85 (1H, d, J=3.0 Hz, H2′); ^{13}C-NMR ($CDCl_3$, 100 MHz) δ: 28.8, 32.6, 70.9, 121.1, 122.6, 126.9, 129.0, 130.9, 136.2, 136.7, 146.1, 150.6, 174.7, 199.8. *Anal.* Calcd for $C_{14}H_{11}IN_2O$: C, 48.0; H, 3.14; N, 8.0. Found: C, 47.97; H, 3.25; N, 8.11.

3-[(3′-Quinolinyl)methylamino]cyclopent-2-en-1-one 14 Compound **10** (600 mg, 2.7 mmol) was added to a suspension of sodium hydride (1 g, 25 mmol, 60% in mineral oil) in anhydrous toluene (50 ml). The mixture was refluxed under nitrogen for 2 h and cooled to room temperature. Methyl iodide (5 ml, 80 mmol) was then added and the mixture was refluxed for 4 h. After cooling, toluene was washed with water and the insoluble residue in the balloon flask was dissolved in dichloromethane and also washed with water. The organic layers were dried over sodium sulfate and evaporated *in vacuo*. The crude product was chromatographed on alumina gel with dichloromethane and a gradient of methanol as eluent to give **14** as a yellow oil: 60%. ^{1}H-NMR ($CDCl_3$, 100 MHz) δ: 2.36 (2H, m, H5), 2.50 (2H, m, H4), 3.41 (3H, s, CH_3), 5.16 (1H, s, H2), 7.56 (1H, m, H7′), 7.71 (1H, m, H6′), 7.78 (1H, d, J=8.1 Hz, H5′), 7.99 (1H, d, J=2.4 Hz, H4′), 8.07 (1H, d, J=8.4 Hz, H8′), 8.76 (1H, d, J=2.5 Hz, H2′); ^{13}C-NMR ($CDCl_3$, 100 MHz) δ: 28.8, 34.8, 41.8, 103.9, 128.1, 128.1, 128.1, 129.7, 130.5, 132.4, 138.5, 147.1, 149.1, 177.3, 204.7. *Anal.* Calcd for $C_{15}H_{14}N_2O$: C, 75.63; H, 5.88; N, 11.76. Found: C, 75.75; H, 5.82; N, 11.81.

3-[(6′-Quinolinyl)methylamino]cyclopent-2-en-1-one 15 This compound was obtained in 60% yield according to the procedure described for compound **14**. Yellow oil. ^{1}H-NMR ($CDCl_3$, 100 MHz) δ: 2.41 (2H, m, H5), 2.56 (2H, m, H4), 3.44 (3H, s, CH_3), 5.21 (1H, s, H2), 7.46 (1H, m, H3′), 7.57 (1H, d, J=6.5 Hz, H7′), 7.67 (1H, s, H5′), 8.15 (2H, m, H4′ and H8′), 8.95 (1H, d, J=2.6 Hz, H2′); ^{13}C-NMR ($CDCl_3$, 100 MHz) δ: 30.1, 34.6, 41.7, 103.6, 122.4, 124.6, 128.2, 128.8, 131.8, 136.3, 143.1, 147.3, 151.5, 177.3, 204.7. *Anal.* Calcd for $C_{15}H_{14}N_2O$: C, 75.63; H, 5.88; N, 11.76. Found: C, 75.60; H, 5.81; N, 11.72.

7-Methyl-10-oxocyclopentano[4,5]pyrrolo[2,3-*c*]quinoline 16 Compound **14** (500 mg, 2.1 mmol) underwent irradiation under different conditions (see Table 3). Then, the solvents were evaporated under reduced pressure. The crude product was chromatographed on alumina gel using dichloromethane as eluent to give **16** as a white powder: 50% (best yield), mp: 213—215 °C. ^{1}H-NMR ($CDCl_3$, 100 MHz) δ: 2.86 (2H, m, H9), 2.95 (2H, m, H8), 3.63 (3H, s, CH_3), 7.60 (2H, m, H2 and H3), 8.10 (1H, m, H4), 8.76 (2H, m, H1 and H6); ^{13}C-NMR ($CDCl_3$, 100 MHz) δ: 20.1, 31.4, 41.3, 121.1, 123.3, 124.2, 127.2, 127.6, 128.2, 129.1, 135.5, 135.6, 143.7, 167.9, 195.3. IR cm^{-1}= $\nu_{C=O}$=1674. *Anal.* Calcd for $C_{15}H_{12}N_2O$: C, 76.27; H, 5.08; N, 11.86. Found: C, 76.16; H, 5.11; N, 11.91.

7-Methyl-10-oxocyclopentano[4,5]pyrrolo[3,2-*f*]quinoline 17 This compound was obtained in 25% yield (best yield) according to the procedure described for compound **16**. White powder, mp: 206—208 °C. ^{1}H-NMR ($CDCl_3$, 100 MHz) δ: 2.71 (2H, m, H9), 2.81 (2H, m, H8), 3.42 (3H, s, CH_3), 7.36 (1H, d, J=9.1 Hz, H6), 7.41 (1H, m, H2), 7.78 (1H, d, J=9.1 Hz, H5), 8.79 (1H, s, H3), 9.17 (1H, dd, J=7.1, 1.22 Hz, H1); ^{13}C-NMR ($CDCl_3$, 100 MHz) δ: 21.3, 31.2, 41.4, 114.7, 119.9, 121.7, 121.8, 123.8, 125.8, 136.1, 139.3, 145.8, 149.2, 166.5, 195.7. IR cm^{-1}= $\nu_{C=O}$=1667. *Anal.* Calcd for $C_{15}H_{12}N_2O$: C, 76.27; H, 5.08; N, 11.86. Found: C, 76.32; H, 5.06; N, 11.80.

10-Hydroxyimino-7-methyl-cyclopentano[4,5]pyrrolo[2,3-*c*]quinoline 18 Compound **16** (180 mg, 0.76 mmol) and hydroxylamine hydrochloride (1 g, 14.4 mmol) were dissolved in absolute ethanol (15 ml) and 2 ml of pyridine were added. The reaction mixture was refluxed under nitrogen for 3 h. After cooling, 30 ml of an aqueous solution of sodium carbonate10% were added. After extraction with dichloromethane, the organic layers were dried over sodium sulfate and evaporated *in vacuo*. The crude product was chromatographed on silica gel using dichloromethane and a gradient of methanol as eluent to give **18** as a white powder: 30%, mp: 154—156 °C. ^{1}H-NMR ($CDCl_3$, 400 MHz) δ: 2.99 (2H, m, H9), 3.32 (2H, m, H8), 3.81 (3H, s, CH_3), 7.52 (2H, m, H2 and H3), 8.0 (1H, d, J=8.0 Hz, H4), 8.78 (1H, s, H6), 8.98 (1H, d, J=8.0 Hz, H1); ^{13}C-NMR ($CDCl_3$, 400 MHz) δ: 22.6, 31.2, 31.7, 116.9, 123.7, 123.7, 126.5, 127.4, 127.5, 128.2, 135.1, 135.6, 143.2, 158.0, 158.3. IR cm^{-1}= $\nu_{C=N}$=1584, ν_{O-H}=2400—3600, MS=252 (35%), 251 (20%), 154 (100%), 136 (80%). *Anal.* Calcd for $C_{15}H_{13}N_3O$: C, 71.71; H, 5.18; N, 16.73. Found: C, 71.83; H, 5.21; N, 16.67.

10-Hydroxyimino-7-methyl-cyclopentano[4,5]pyrrolo[3,2-*f*]quinoline 19 This compound was obtained in 33% yield according to the procedure described for compound **18**. White powder, mp: 155—157 °C. ^{1}H-NMR ($CDCl_3$, 400 MHz) δ: 3.09 (2H, m, H9), 3.27 (2H, m, H8), 3.85 (3H, s, CH_3), 7.75 (1H, d, J=9.1 Hz, H6), 7.85 (1H, m, H2), 8.15 (1H, d, J=9.1 Hz , H5), 8.85 (1H, d, J=5.4 Hz, H3), 10.07 (1H, d, J=8.3 Hz, H1); ^{13}C-NMR ($CDCl_3$, 400 MHz) δ: 24.2, 31.5, 31.7, 113.9, 117.3, 118.6, 121.3, 121.8, 125.6, 136.6, 139.9, 141.7, 145.8, 158.1, 159.01. IR cm^{-1}= $\nu_{C=N}$=1586, ν_{O-H}=2400—3600, MS=252 (5%), 250 (6%), 154 (40%), 149 (100%), 136 (40%). *Anal.* Calcd for $C_{15}H_{13}N_3O$: C, 71.71; H, 5.18; N, 16.73. Found: C, 71.63; H, 5.20; N, 16.69.

8,9-Dihydro-7-methyl-11-oxopyrido[3′,4′-4,5]pyrrolo[2,3-*c*]quinoline 22 Compound **18** (80 mg, 0.32 mmol) was added to polyphosphoric acid PPA (3 g) and phosphorus pentoxide (2 g) and the reaction mixture was stirred at 130 °C for 1 h. After cooling, 100 ml of ice cooled water were added. Then, sodium carbonate was added until pH became 7. After extraction with dichloromethane, the organic layers were dried over sodium sulfate and evaporated *in vacuo*. The crude product was chromatographed on silica gel using dichloromethane and a gradient of methanol as eluent to give **22** as a white powder: 30%, mp: 184—186 °C. ^{1}H-NMR ($CDCl_3$, 400 MHz) δ: 3.37 (2H, t, J=7.0 Hz, H8), 3.94 (2H, t, J=7.0 Hz, H9), 4.14 (3H, s, CH_3), 7.97 (2H, m, H2 and H3), 8.23 (1H, d, J=8.1 Hz, H4), 9.50 (1H, s, H6), 9.78 (1H, d, J=8.4 Hz, H1); ^{13}C-NMR ($CDCl_3$, 400 MHz) δ: 22.3, 32.1, 39.7, 109.02, 120.8, 122.3, 129.6, 129.9, 130.0 (2C), 132.4, 133.5, 134.2, 156.1, 167.0. IR cm^{-1}=$\nu_{C=O}$=1645, MS=252 (18%), 154 (96%), 136 (100%). *Anal.* Calcd for $C_{15}H_{13}N_3O$: C, 71.71; H, 5.18; N, 16.73. Found: C, 71.79; H, 5.22; N, 16.79.

8,9-Dihydro-7-methyl-11-oxo-pyrido[3′,4′-4,5]pyrrolo[3,2-*f*]quinoline 23 This compound was obtained in 60% yield according to the procedure described for compound **22**. White powder, mp: 198—200 °C. ^{1}H-NMR ($CDCl_3$, 400 MHz) δ: 3.29 (2H, t, J=7.1 Hz, H8), 3.89 (2H, t, J=7.1 Hz, H9), 4.02 (3H, s, CH_3), 7.98 (1H, m, H2), 8.16 (1H, d, J=9.2 Hz, H6), 8.26 (1H, d, J=9.2 Hz, H5), 8.98 (1H, d, J=5.0 Hz, H3), 10.81 (1H, d, J=8.5 Hz, H1); ^{13}C-NMR ($CDCl_3$, 400 MHz) δ: 21.9, 31.4, 40.2, 107.8, 116.3, 120.2, 120.3, 121.1, 124.7, 135.5, 136.5, 140.9, 147.3, 148.3, 168.1. IR cm^{-1}=$\nu_{C=O}$=1639, MS=252 (6%), 250 (3%), 154 (100%), 149 (60%), 136 (85%). *Anal.* Calcd for $C_{15}H_{13}N_3O$: C, 71.71; H, 5.18; N, 16.73. Found: C, 71.59; H, 5.13; N, 16.68.

11-Hydroxy-7-methyl-pyrido[3′,4′-4,5]pyrrolo[2,3-*c*]quinoline 26 Compound **22** (40 mg, 0.16 mmol) was added to a supension of Pd/carbon 10% (100 mg) in diphenylether (20 ml) and the reaction mixture was refluxed under nitrogen for 1 h. Still hot, the reaction mixture was filtered in order to eliminate Pd/carbon, which was washed with a hot mixture of dichloromethane and methanol (50/50). Dichloromethane and methanol were evaporated *in vacuo* and the diphenylether containing the final product was chromatographed on silica gel using dichloromethane as eluent to eliminate diphenylether and dichloromethane and a gradient of methanol to give **26** as a yellow oil: 30%. ^{1}H-NMR ($CDCl_3$, 400 MHz) δ: 4.01 (3H, s, CH_3), 6.91 (1H, d, J=7.1 Hz, H8), 7.77 (1H, d, J=6.9 Hz, H9), 7.90 (2H, m, H2 and H3), 8.18 (1H, d, J=8.0 Hz, H4), 9.43 (1H, s, H6), 10.1 (1H, d, J=8.4 Hz, H1); ^{13}C-NMR ($CDCl_3$, 400 MHz) δ: 31.2, 96.6, 110.5, 119.0, 120.1, 123.5, 129.3, 129.8, 130.6, 131.4, 132.8, 133.3, 134.2, 136.7, 152.20. IR cm^{-1}=ν_{O-H}=2800—3600, MS=250 (100%), 176 (30%), 154 (35%), 136 (34%). *Anal.* Calcd for $C_{15}H_{11}N_3O$: C, 72.29; H, 4.42; N, 16.87. Found: C, 72.39; H, 4.44; N, 16.87.

11-Hydroxy-7-methyl-pyrido[3′,4′-4,5]pyrrolo[3,2-*f*]quinoline 27 This compound was obtained in 32% yield according to the procedure described for compound **26**. Yellow oil. ^{1}H-NMR ($CDCl_3$, 400 MHz) δ: 3.87 (s, 3H, CH_3), 6.54 (d, 1H, J=7.1 Hz, H8), 7.27 (d, 1H, J=7.1 Hz, H9), 7.5 (m, 2H, H2 and H3), 7.76 (d, 1H, J=9.1 Hz, H6), 8.0 (d, 1H, J=9.1 Hz, H5), 8.74 (m, 1H, H1); ^{13}C-NMR ($CDCl_3$, 400 MHz) δ: 30.3, 94.3, 114.1, 119.3, 121.2, 124.9, 126.7, 127.2, 130.8, 131.4, 136.0, 138.0, 145.2, 146.0, 147.7. IR cm^{-1}=ν_{O-H}=2800—3600, MS=250 (100%), 176 (40%), 154 (65%), 136 (45%). *Anal.* Calcd for $C_{15}H_{11}N_3O$: C, 72.29; H, 4.42; N, 16.87. Found: C, 72.38; H, 4.46; N, 16.91.

Biological Assay Doxorubicine hydrochloride (Pharmacia, St-Quentin en Yvelines, France), RPMI 1640 medium and fetal calf serum (Polylabo, Paris, France) were used in this study. 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) was provided by Sigma (St-Quentin Fallavier, France). All other reagents were of analytical grade and were obtained from commercial sources.

Cells and Culture: The human leukemic cell line K 562 was obtained from the American type culture collection (Rockville, MD, U.S.A.). The human ovarian carcinoma cell line A 2780 was generously given by Dr P. Canal (Centre Claudius Regaud, Toulouse). The doxorubicine resistant cell line A 2780 R was established by the continuous exposure of cells to gradually increasing concentrations of doxorubicine and was maintained in a medium supplemented with doxorubicin at 0.1 μg/ml. The MDR phenotype expression of A 2780 R cell lines was assessed by an immunohistochemistry method, using the two P-glycoprotein specific murine monoclonal antibody C219 (Cantocor, Malvern, PA, U.S.A.) and JSB1 (Tebu, Le Perray en Yvelines, France). Cultures were grown in RPMI 1460 medium supplemented with 10% fetal calf serum, antibiotics and glutamine at 37 °C in a humidified atmosphere containing 5% CO_2.

Cytotoxicity Assay: In all the experiments, K 562 and A 2780 cells were seeded at a final density of 5000 cells/well in 96 well microtiter plates and were treated with drugs (doxorubicine and compound **16**, **17**, **22**, **23**, **26**, **27**). Seven dilutions were used for each drug. After 96 h of incubation, 10 μl of MTT solution in PBS (5 mg/ml, Phosphate-buffer saline pH 7.3) were added to each well and the wells were exposed to 37 °C for 4 h. This colorimetric assay is based on the ability of live and metabolically unimpaired tumor-cell targets to reduce MTT to a blue formazan product. Then, 100 μl of a mixture of isopropanol and 1 M hydrochloric acid (96/4, v/v) were added to each well. After 10 min of vigourous shaking so as to solubilize formazan crystals, the absorbance was measured on a microculture plate reader (Dynatech MR 5000, France) at 570 nm. For each assay, at least three experiments were performed in triplicate. The resistance factor was calculated from the ratio between the IC_{50}% growth-inhibitory concentrations (IC_{50} values) recorded from A 2780 R and A 2780 S cells, respectively, for the following tested drugs: doxorubicine, compounds **16**, **17**, **23**, **26**, **27**.

References

1) Goodwin S., Smith A. F., Horning E. C., *J. Am. Chem. Soc.*, **81**, 1903—1906 (1959).
2) Chu Y., Hsu M. T., *Nucleic Acids Res.*, **20**, 4033—4038 (1992).
3) Monnot M., Mauffret O., Simon V., Lescot E., Psaume B., Saucier J. M., Charra M., Belehradek J., Fermandjian S., *J. Biol. Chem.*, **25**, 1820—1829 (1991).
4) Nguyen C. H., Lhoste J. M., Lavelle F., Bissery M. C., Bisagni E., *J. Med. Chem.*, **33**, 1519—1528 (1990).
5) Nabiev I., Chourpa I., Riou J. F., Nguyen C. H., Lavelle F., Manfait M., *Biochemistry*, **33**, 9013—9023 (1994).
6) Riou J. F., Fossé P., Nguyen C. H., Kragh Larsen A., Bissery M. C., Grondard L., Saucier J. M., Bisagni E., Lavelle F., *Cancer Res.*, **53**, 5987—5993 (1993).
7) Da Settimo A., Da Settimo F., Marini A. M., Primofiore G., Salerno S., Viola G., Dalla Via L., Magno S. M., *Eur. J. Med. Chem.*, **33**, 685—696 (1998).
8) Dalla Via L., Gia O., Magno S. M., Da Settimo A., Primofiore G., Da Settimo F., Simorini F., Marini A. M., *Eur. J. Med. Chem.*, **37**, 475—486 (2002).
9) Dupuy M., Pinguet F., Chavignon O., Chezal J. M., Teulade J. C., Chapat J. P., Blache Y., *Chem. Pharm. Bull.*, **49**, 1061—1065 (2001).
10) Dupuy M., Blache Y., Bailly C., Poujol S., Chapat J. P., Pinguet F., *Anticancer Res*., **22**, 3365—3371 (2002).
11) Aragon P. J., Chezal J. M., Chavignon O., Teulade J. C., Blache Y., *Heterocycles*, **60**, 551—561 (2003).
12) Blache Y., Benezech V., Chezal J. M., Boule P., Viols H., Chavignon O., Teulade T. C., Chapat J. P., *Heterocycles*, **53**, 905—916 (2000).
13) Aragon P. J., Chezal J. M., Chavignon O., Teulade J. C., Chapat J. P., Blache Y., *Heterocyclic Commun.*, **9**, 189—194 (2003).
14) Gardette D., Gramain J. C., Lepage M. E., Troin Y., *Can. J. Chem.*, **67**, 213—219 (1989).
15) Sekar M., Vanitha S., Rajendra Prasad K. J., *Zeitschrift fuer Naturforschung B: Chemical Sciences*, **49**, 687—690 (1994).
16) Scheiber P., Nemes P., *Heterocycles*, **41**, 2189—2194 (1995).
17) Jeyaraman R., Senthilkumar U. P., *Tetrahedron Lett.*, **35**, 9279—9280 (1994).
18) Shanmugasundaram K., Rajenda Prasad K. J., *Heterocycles*, **51**, 2161—2169 (1999).