Eur J Med Chem (1993) 28, 721–726

In vitro cytotoxicity of carbazole derivatives IV. 5,11-Dimethyl-6*H*-pyrido[3,2-*b*]carbazoles substituted on the pyridine ring

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(Received 20 January 1993; accepted 10 May 1993)

Summary — A series of 5,11-dimethyl-6*H*-pyrido[3,2-*b*]carbazoles, structurally related to the antitumor drug ellipticine, were synthesized from 3-amino-1,4-dimethyl-9*H*-carbazole. Among 17 derivatives, bearing various substituents on the pyridine ring, and preliminarily evaluated for cytotoxicity on L1210-cultured cells, 13 (76%) were found to be active. One of them, 5,11-dimethyl-4-ethoxy-6*H*-pyrido[3,2-*b*]carbazole, although non-substituted on C-9, displayed an activity similar to that of 9-hydroxy-*N*-2-methyl-ellipticinium acetate. Structure–activity relationships have been described in detail.

cytotoxicity / leukemia L1210 / clonogenic assay / pyrido[3,2-*b*]carbazoles

Introduction

As a result of the antitumoral properties [1–7] of ellipticine (6*H*-pyrido[4,3-*b*]carbazole), methoxyellipticine and their derivatives, many pyrido-carbazole syntheses have been described, particularly those regarding the obtention of 10*H*-pyrido[3,4-*b*]- and [2,3-*b*]-carbazoles [8–11], and 7*H*-pyrido[3,2-*c*]-, [4,3-*c*]-, [3,4,*c*]- and [2,3-*c*]carbazoles [12–14] while the isomeric 6*H*-pyrido[3,2-*b*]carbazole was not conspicuous. In a previous paper [15], we described a 3-amino-1,4-dimethyl-9*H*-carbazole **5** synthesis leading to 5,11-dimethyl-6*H*-pyrido[3,2-*b*]carbazoles. In the present paper, the cytotoxic activity of these pyridocarbazoles on L1210 leukemia is reported and compared with that of *N*2-methyl-9-hydroxy-ellipticinium acetate (NMHE).

Chemistry

Diethyl ethoxymethylidene malonate reacted with 3-amino-1,4-dimethyl-9*H*-carbazole **5** to provide the isolatable intermediary compound **6** which after cyclization led either to 3-carbethoxypyridone **8** by heating in diphenyl ether or to 4-ethoxypyridone **7** *via in vacuo* sublimation at 200°C. The 3-carbethoxypyridone **8** furnished the 4-chloropyridocarbazole **9a** after treatment with phosphorus oxychloride. The amine **5** also reacted with diethyl malonate to give the stable amido ester **10** which after cyclization led to hydroxypyridone **11** (scheme 1).

Condensation of substituted ethyl acetoacetates with amine **5** led to intermediary β-keto-esters such as **12** which could be directly cyclized in diphenyl ether to yield the pyridocarbazolones **13a–g**. The pyridocarbazolones **13c** and **13e** led to 4-chloro-pyridocarbazoles **9b–c** after treatment with phosphorus oxychloride. An X-ray cristallographic study of lactam–lactim tautomeric forms of 5,11-dimethyl-6*H*-pyrido[3,2-*b*]carbazoles clearly showed that the solid existed in the lactam form [16], while in solution (CH_3CN) the OH absorption indicated that the lactim form was prevalent. This was confirmed by acetylation of derivatives **13a–f** which, when treated with acetic anhydride and acetic acid, led to the acetoxy derivatives **14a–b**. Because some of these compounds were not very soluble, their salts **9d**, **14c**, **15a** and **15b** were prepared from oxalic, tartaric or sulfuric acids.

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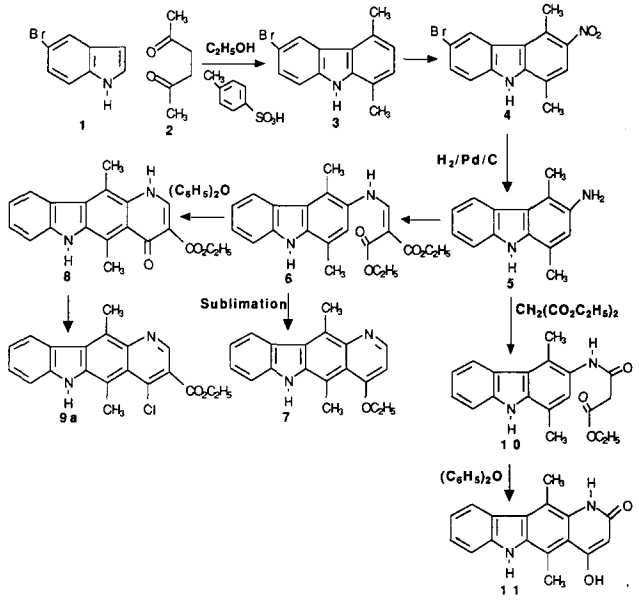


Scheme 1.

Biological data

Results and discussion

The derivatives were evaluated *in vitro* against L1210 murine leukemia. The results are presented in table I, together with those obtained for the tricyclic analogue 3-amino-1,4-dimethyl-6*H*-carbazole and for *N*2-methyl-9-hydxoxy-ellipticinium (NMHE). Among the 17 5,11-dimethyl-pyrido[3,2-*b*]carbazoles assayed, 13 were found to be cytotoxic. Using continuous exposure, the 10 compounds **7**, **8**, **9a**, **13a–d**, **13f**, **14a–b** totally inhibited colony formation when assayed at 10 μg/ml. At 1 μg/ml, compound **7** induced an 80% reduction in colony number (fig 1). When the contact between cells and the drugs was reduced to 1 h incubation before plating, compounds **7**, **8** and **9a** remained fully active at 10 μg/ml, while **13a–b** and **13f** were still able to reduce colony formation by > 50% at this concentration.

From the structure–activity point of view, certain features can be noted:

Table I. *In vitro* cytotoxicity of 5,11-dimethyl-6*H*-pyrido[3,2-*b*]-carbazoles, expressed as the reduction in colony formation by L1210 cells cultivated in soft agar.

Compound No	*Colony formation (% of control)*						*Activity*	
	Continuous exposure			*1 h exposure*			*EC*	*EB*
	0.1 μg/ml	*1 μg/ml*	*10 μg/ml*	*0.1 μg/ml*	*1 μg/ml*	*10 μg/ml*		
5	67 ± 2	46 ± 3	0 ± 0	93 ± 2	80 ± 3	45 ± 3	+++	±
6	100 ± 2	95 ± 2	47 ± 3	100 ± 2	99 ± 2	96 ± 4	±	–
7	83 ± 4	21 ± 1	0 ± 0	96 ± 2	83 ± 3	0 ± 0	+++	++
8	98 ± 5	93 ± 6	0 ± 0	99 ± 2	85 ± 5	0 ± 0	++	++
9a	91 ± 2	64 ± 3	0 ± 0	98 ± 4	83 ± 4	0 ± 0	++	++
9b	97 ± 1	92 ± 3	88 ± 3	99 ± 2	94 ± 3	88 ± 4	–	–
9c	94 ± 3	81 ± 2	30 ± 2	101 ± 1	94 ± 1	85 ± 1	±	–
11	98 ± 3	98 ± 3	95 ± 2	97 ± 2	96 ± 3	97 ± 3	–	–
13a	96 ± 2	72 ± 4	0 ± 0	97 ± 2	87 ± 2	25 ± 2	++	+
13b	100 ± 2	81 ± 2	0 ± 0	100 ± 2	99 ± 2	40 ± 2	++	±
13c	95 ± 5	84 ± 3	0 ± 0	99 ± 3	95 ± 3	90 ± 2	++	–
13d	89 ± 2	73 ± 2	0 ± 0	97 ± 3	84 ± 3	64 ± 2	++	–
13e	97 ± 4	90 ± 2	62 ± 4	98 ± 3	94 ± 3	83 ± 4	–	–
13f	90 ± 3	75 ± 3	0 ± 0	95 ± 2	83 ± 3	38 ± 1	+	±
13g	97 ± 1	93 ± 2	82 ± 3	97 ± 3	91 ± 2	81 ± 2	–	–
14a	99 ± 3	92 ± 2	0 ± 0	98 ± 1	95 ± 3	83 ± 1	++	–
14b	97 ± 3	83 ± 2	0 ± 0	98 ± 2	95 ± 3	49 ± 3	++	±
14c	100 ± 3	81 ± 3	22 ± 3	99 ± 1	87 ± 2	47 ± 3	+	±
NMHE	85 ± 3	28 ± 2	0 ± 0	98 ± 3	78 ± 2	0 ± 0	+++	++

–: Inactive at all doses (colony number > 50% of control); ±: weakly active at 10 μg/ml (colony number between 50–30% of control); +: active at 10 μg/ml (colony number < 30% of control); ++: fully active at 10 μg/ml (total inhibition of colony formation); +++: active at 1 μg/ml (colony number < 50% of control); EC: continuous exposure; EB: 1-h exposure; NT: not tested.

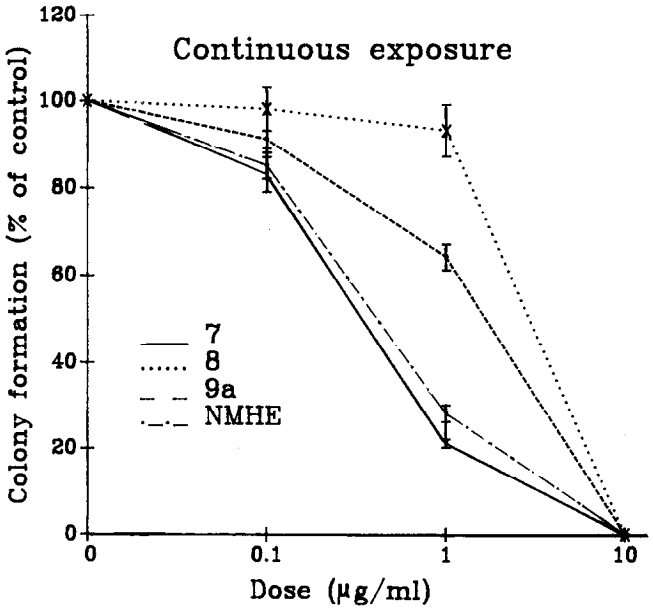


Fig 1. Colony inhibition after continuous exposure of L1210 cells with compounds **7**, **8** and **9a**. Comparison with the results obtained with NMHE.

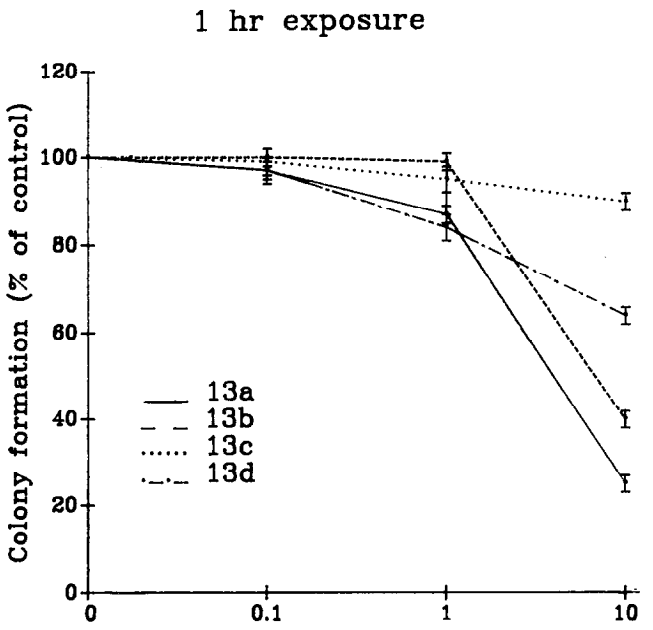


Fig 2. Colony inhibition after 1 h exposure of L1210 cells with compounds **13a–d**: influence of alkyl substitution on C-2.

– influence of substitution on C-2: for 5,11-dimethyl-pyrido[3,2-*b*]carbazolones bearing a 2-alkyl side chain, cytotoxicity decreased as the number of carbon units in the side chain increased. This clearly appeared when the 1 h exposure protocol was used (table I, fig 2). Likewise, replacing the 2-methyl group by 2-methoxymethyl led to **13e**, a compound which is only weakly active after continuous exposure;
– influence of substitution on C-3: whereas the cytotoxicity of 2,5,11-trimethyl-pyrido[3,2-*b*]carbazolone **13a** remained unchanged upon addition of a methyl group on C-3 **13f**, it was completely abolished by addition of a benzyl group **13g** (table I, fig 3). The presence of an ethoxycarbonyl group on C-3 seemed favourable to activity, since compounds **8** and **9a** were among the most active 6*H*-pyrido[3,2-*b*]carbazoles presented here (table I);
– influence of substitution on C-4: acetylation of the 4-hydroxyl group of **13a** and **13f** led to slightly less cytotoxic derivatives **14a** and **14b**. On the other hand, compound **7** was more active than **13a** and **13f**, and this might mainly be due to the ethylation of the 4-hydroxyl group, as far as the results discussed above suggest that the absence of methyl group on the pyrido ring is not likely to account for such an increase of activity. Replacing the 4-hydroxyl group of **8** by a chlorine atom **9a** caused a slight increase in activity. This was also the case for the 2-methoxy-methyl derivatives **13e** and **9c** but the opposite was observed for their 2-propyl isomers **13c** and **9b**;

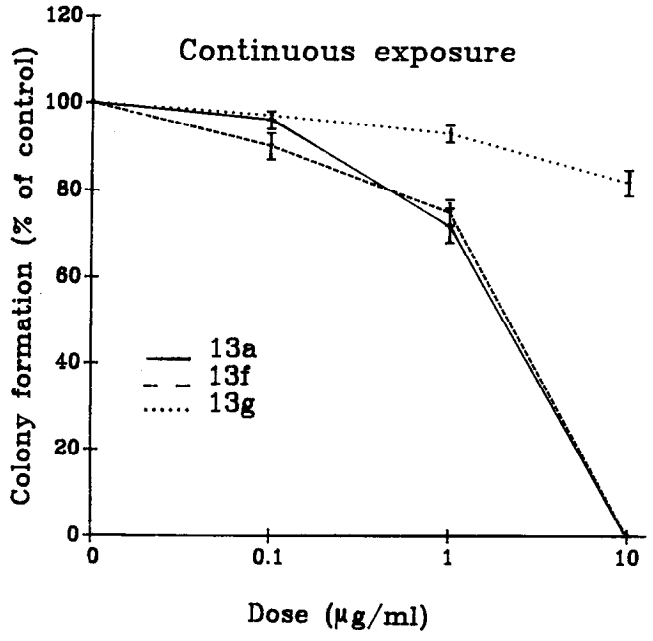
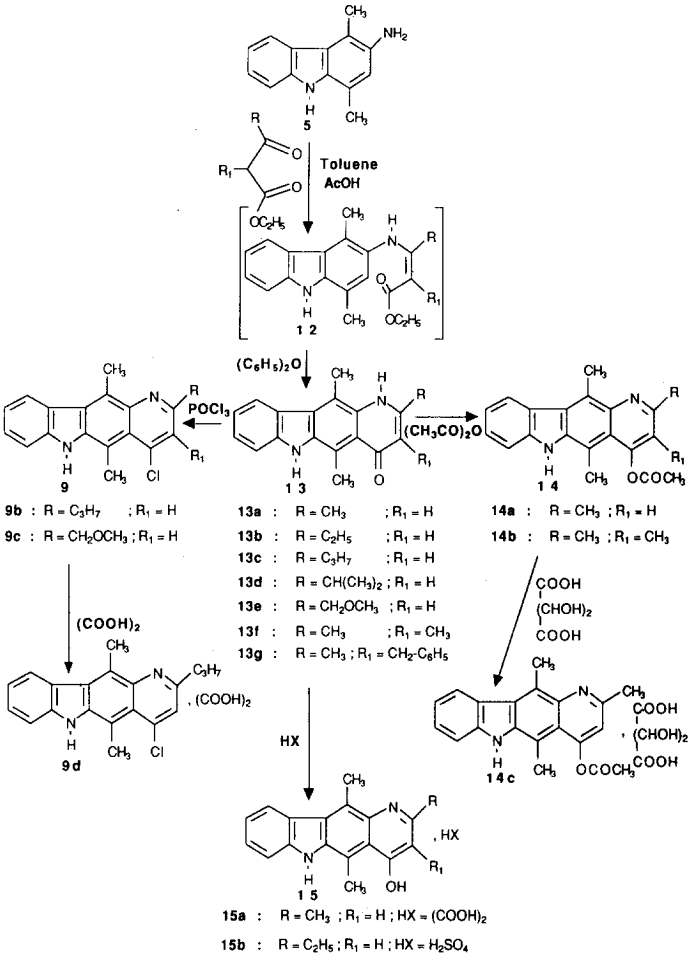


Fig 3. Colony inhibition after continuous exposure of L1210 cells with compounds **13a** and **13f–g**: influence of alkyl substitution on C-3.



Scheme 2.

– influence of the pyridine ring: under continuous exposure, similar levels of cytotoxicity were observed for the active 5,11-dimethyl-pyrido[3,2-*b*]carbazoles presented here and their tricyclic precursor **5**. On the other hand, the uncyclized intermediary **6** and the hydroxypyridone **11** (lacking the aromatisation of the pyrido ring) were not cytotoxic.

Conclusion

The 5,11-dimethyl-6*H*-pyrido[3,2-*b*]carbazoles is an extremely interesting series since 13 of the 17 derivatives are cytotoxic in L1210 leukemia cell culture. These tetracyclic compounds have to be compared with their tricyclic analogues (1,4-dimethyl-9*H*-carbazole) [15] as well as with the ellipticines in order to obtain a better knowledge on structure–activity relationships. The present study is limited to the derivatives which are not substituted on C-9. It would be helpful to study the influence of this substitution since, in a closely related series, the derivatives bearing a hydroxyl group in this position are the most active [15, 17–21]. The cytotoxicity studies will be continued with the 5,11-dimethyl-pyrido[3,2-*b*]carbazole derivatives that are not substituted on C-4.

The most active compounds presented here are currently being tested *in vivo* for antineoplastic activity. We are also studying the interaction of 5,11-dimethyl-6*H*-pyrido[3,2-*b*]carbazoles with DNA and/or with the DNA-topoisomerase II complex [22, 23] in order to contribute to the identification of their cellular targets and help in the synthesis of more selective derivatives.

Experimental protocols

Chemical synthesis

Melting points were determined on a Kofler type WME apparatus and are uncorrected. IR spectra were recorded on a Philips PU spectrometer. ^{1}H-NMR spectra were recorded on a Varian EM 390 spectrometer at 90 MHz in hexadeuteriodimethylsulfoxide with tetramethylsilane as internal reference. Chemical shifts are expressed as δ (ppm) relative to TMS. Elemental analyses were in agreement with the proposed structures within ± 0.4% of theoretical values. ^{1}H-NMR and IR spectra data of compounds **6–8, 13a, 13c, 13d, 13f, 13g** and **14a** have been described in a preliminary communication [24].

*Ethyl-2-carbethoxy-3-(1,4-dimethyl-9H-carbazolyl-3-amino) acrylate **6***

To a solution of 5 g (0.024 mol) 3-amino-1,4-dimethyl-9*H*-carbazole **5** in 60 ml in dry ethanol was added 6 ml (0.0288 mol) of diethylethoxymethylene malonate. The mixture was stirred at 80°C for 1 h. The solid was filtered off and crystallized from acetonitrile to give 7.25 g (75%) of **6**, mp: 230°C.

*5,11-Dimethyl-4-ethoxy-6H-pyrido[3,2]carbazole **7***

The sublimation *in vacuo* at 200°C of 1 g (0.0026 mol) ethyl-2-carbethoxy-3-(1,4-dimethyl-9*H*-carbazolyl-3-amino) acrylate **6** afforded 0.2 g of yellow crystals of compound **7** (26%), mp: 264°C.

*3-Carbethoxy-1,4-dihydro-5,11-dimethyl-4-oxo-6H-pyrido[3,2-b]-carbazole **8***

Ethyl-2-carbethoxy-3-(1,4-dimethyl-9*H*-carbazolyl-3-amino) acrylate **6** (1 g, 0.0026 mol) in 20 ml diphenyl ether was stirred at 240°C for 20 min. On cooling, a precipitate formed which was filtered and washed successively with diethyl ether and ice-cold ethanol. The orange product was recrystallized (acetonitrile) to give 0.40 g (44%) of **8**, mp: > 270°C.

*1,4-Dimethyl-3-ethylmalonamido-9H-carbazole **10***

A mixture of 1 g (0.0048 mol) 3-amino-1,4-dimethyl-9*H*-carbazole **5** in 1.10 ml (0.0072 mol) diethyl malonate was stirred at 130°C for 30 min. The solid was filtered off and crystallized from acetonitrile to yield 0.75 g (48%) of **10**, mp: 240°C.

IR: 3380, 3270 (2NH); 1730, 1640 (CO). ^{1}H-NMR (DMSO–d_6) δ: 11.16, 9.66 (2s, 2H, 2NH); 7.00 (s, 1H, H_2); 8.13 (d, 1H, H_5); 7.43 (m, 3H, $H_{6/7/8}$); 4.10 (q, 2H, CH_2CH_3); 3.40 (s, 2H, CH_2); 2.66, 2.46 (2s, 6H, $2CH_3$); 1.23 (t, 3H, CH_2CH_3). Anal $C_{19}H_{20}N_2O_3$ (C, H, N).

1,2-Dihydro-5,11-dimethyl-4-hydroxy-2-oxo-6H-pyrido[3,2-b]-carbazole ***11***
Prepared analogously to **8**, yield: 23%; yellow solid, mp: 265°C. IR: 3400, 3330 (2NH); 1650 (CO). ^{1}H-NMR (DMSO–d_6) δ: 11.06, 9.60 (2s, 2H, 2NH); 3.50 (s, 1H, OH); 7.30, 6.95 (2m, 4H, $H_{7/8}$, $H_{9/3}$); 8.06 (d, 1H, H_{10}); 2.56, 2.40 (2s, 6H, $2CH_3$). Anal $C_{17}H_{14}N_2O_2$ (C, H, N).

1,4-Dihydro-4-oxo-2,5,11-trimethyl-6H-pyrido[3,2-b] carbazole ***13a****: general procedure*
A solution of 1 g (0.0048 mol) 3-amino-1,4-dimethyl-9*H*-carbazole **5** in a mixture of 300 ml benzene, 0.90 ml (0.0072 mol) ethyl acetoacetate and 0.50 ml acetic acid was refluxed for 3 h. The solution was removed *in vacuo*. The oily residue was added in 10 ml diphenyl ether and heated with stirring at 240°C for 20 min. When the solution was diluted in diethyl ether, the precipited product was filtered off and recrystallized from acetonitrile to give 0.80 g (60%) of **13a**, mp: ≤ 270°C.

1,4-Dihydro-5,11-dimethyl-2-ethyl-4-oxo-6H-pyrido[3,2-b]-carbazole ***13b***
Prepared analogously to **13a**, yield: 57%; yellow solid, mp > 270°C. IR: 3440, 3200 (2NH); 1620 (CO). ^{1}H-NMR (DMSO–d_6) δ: 10.96, 9.83 (2s, 2H, 2NH); 5.73 (s, 1H, H_3); 7.43, 7.10 (2m, 3H, $H_{7/8}$, H_9); 8.23 (d, 1H, H_{10}); 3.06, 2.90 (2s, 6H, $2CH_3$); 2.80 (q, 2H, CH_2CH_3); 1.23 (t, 3H, CH_2CH_3). Anal $C_{19}H_{18}N_2O$ (C, H, N).

1,4-Dihydro-5,11-dimethyl-4-oxo-2-propyl-6H-pyrido[3,2-b]-carbazole ***13c***
Prepared analogously to **13a**, yield: 54%; yellow solid, mp: > 270°C.

1,4-Dihydro-5,11-dimethyl-4-oxo-2-isopropyl-6H-pyrido[3,2-b]-carbazole ***13d***
Prepared analogously to **13a**, yield: 27%; yellow solid, mp: > 270°C.

1,4-Dihydro-5,11-dimethyl-2-methoxymethyl-4-oxo-6H-pyrido[3,2-b]carbazole ***13e***
Prepared analogously to **13a**, yield: 51%; yellow solid, mp: > 270°C. IR: 3320, 3200 (2NH); 1610 (CO). ^{1}H-NMR (DMSO–d_6) δ: 11.06, 9.90 (2s, 2H, 2NH); 5.93 (s, 1H, H_3); 7.46, 7.10 (2m, 3H, $H_{7/8}$, H_9); 8.26 (d, 1H, H_{10}); 4.46 (s, 2H, CH_2); 3.36 (s, 3H, OCH_3); 3.06, 2.93 (2s, 6H, $2CH_3$). Anal $C_{19}H_{18}N_2O_2$ (C, H, N).

1,4-Dihydro-4-oxo-2,3,5,11-tetramethyl-6H-pyrido[3,2-b]-carbazole ***13f***
Prepared analogously to **13a**, yield: 37%; yellow solid, mp: > 270°C.

3-Benzyl-1,4-dihydro-4-oxo-2,5,11-trimethyl-6H-pyrido[3,2-b]-carbazole ***13g***
Prepared analogously to **13a**, yield: 57%; yellow solid, mp: > 270°C.

4-Acetoxy-2,5,11-trimethyl-6H-pyrido[3,2-b]carbazole ***14a***
1 g (0.0036 mol) 1,4-dihydro-4-oxo-2,5,11-trimethyl-6*H*-pyrido[3,2-*b*]carbazole **13a** was dissolved in 20 ml acetic acid and 10 ml acetic anhydride and the solution refluxed for 1 h. Workup as above yielded 0.80 g (69%) of yellow crystals, mp: 170°C.

4-Acetoxy-2,3,5,11-tetramethyl-6H-pyrido[3,2-b]carbazole ***14b***
The yield and the reaction conditions for obtaining the 4-acetoxy-2,5,11-trimethyl-6*H*-pyrido[3,2-*b*]carbazole **14b** (49%, mp: 162°C) were the same as for **14a**. IR: 3380 (NH); 1750 (CO). ^{1}H-NMR (DMSO–d_6) δ: 11.02 (s, H, NH); 7.46, 7.13 (2m, 3H, $H_{7/8}$, H_9); 8.30 (d, 1H, H_{10}); 3.06, 2.86, 2.70, 2.50 and 2.16 (5s, 15H, $5CH_3$). Anal $C_{21}H_{20}N_2O_2$ (C, H, N).

3-Carbethoxy-4-chloro-5,11-dimethyl-6H-pyrido[3,2-b]carbazole ***9a***
A mixture of 1 g (0.003 mol) 3-carbethoxy-1,4-dihydro-5,11-dimethyl-4-oxo-6*H*-pyrido[3,2-*b*]carbazole **8**, 3 ml pyridine and 20 ml phosphorus oxychloride was heated at reflux under N_2 for 5 h. The excess $POCl_3$ was removed *in vacuo*, the residue cooled to 5°C and poured into a large excess of water; 30 ml sodium hydroxide at 10% was added and extracted with ethyl acetate. The dried organic extract was filtered and concentrated *in vacuo*. The solid was filtered off and recrystallized from acetonitrile to give 0.60 g (57%) of **9a**, mp: 208°C. IR: 3370 (NH). ^{1}H-NMR (DMSO–d_6) δ: 11.25 (s, H, NH); 8.72 (s, 1H, H_2); 7.53, 7.46 (2m, 3H, $H_{7/8}$, H_9); 8.25 (d, 1H, H_{10}); 4.35 (q, 2H, CH_2); 3.15, 3.04 (2s, 6H, $2CH_3$); 1.35 (t, 3H, CH_3). Anal $C_{20}H_{17}N_2OCl$ (C, H, N, Cl).

4-Chloro-5,11-dimethyl-2-propyl-6H-pyrido[3,2-b]carbazole ***9b***
Prepared analogously to **9a**, yield: 56%; yellow solid, mp: 170°C. IR: 3320 (NH). ^{1}H-NMR (DMSO–d_6) δ: 11.00 (s, H, NH); 7.45, 7.20 (2m, 4H, $H_{7/8}$, $H_{9/3}$); 8.25 (d, 1H, H_{10}); 3.10, 2.95 (2s, 6H, $2CH_3$); 2.65, 1.75 (2m, 4H, $(CH_2)_2$); 1.00 (t, 3H, CH_3). Anal $C_{20}H_{17}N_2Cl$ (C, H, N, Cl).

4-Chloro-5,11-dimethyl-2-methoxymethyl-6H-pyrido[3,2-b]-carbazole ***9c***
Prepared analogously to **9a**, yield: 58%; yellow solid, mp: 150°C. IR: 3320 (NH). ^{1}H-NMR (DMSO–d_6) δ: 11.13 (s, H, NH); 7.50, 716 (2m, 4H, $H_{7/8}$, $H_{9/3}$); 8.23 (d, 1H, H_{10}); 4.60 (s, 2H, CH_2); 3.43 (s, 3H, OCH_3); 3.20, 3.10 (2s, 6H, $2CH_3$). Anal $C_{19}H_{17}N_2OCl$ (C, H, N, Cl).

4-Chloro-5,11-dimethyl-2-propyl-1H+-6H-pyrido[3,2-b]-carbazolium(oxalate) ***9d***
To a solution of 1 g (0.0031 mol) 4-chloro-5,11-dimethyl-2-propyl-6*H*-pyrido[3,2-*b*]carbazole **9b** in 30 ml isopropanol was added oxalic acid 0.56 g (0.062 mol) and the mixture refluxed for 20 min. The solid was filtered off and recrystallized from acetonitrile to yield 0.40 g (31%) of **9d**, mp: 250°C. IR: 3200 (NH); 3340 (OH); 2800, 2710 (COO^-). ^{1}H-NMR (DMSO–d_6) δ: 11.00 (s, H, NH); 7.80, 7.43 and 7.13 (3m, 6H, $H_{7/8}$, $H_{9/3}$, OH, NH^+); 8.20 (d, 1H, H_{10}); 3.20, 3.06 (2s, 6H, $2CH_3$); 2.85, 1.83 (2m, 4H, $(CH_2)_2$); 1.00 (t, 3H, CH_3). Anal $C_{20}H_{18}N_2O_5$ (C, H, N).

4-Acetoxy-2,5,11-trimethyl-1H-6H-pyrido[3,2-b]carbazolium (tartrate) ***14c***
Prepared analogously to **9d**, yield: 30%; yellow solid, mp: 245°C. IR: 3200 (NH); 3340 (OH); 2700, 2400 (COO^-).

^{1}H-NMR (DMSO–d_6) δ: 11.00 (s, H, NH); 5.83 (s, 1H, H_3); 7.45, 7.15 (2m, 3H, $H_{7/8}$, H_9); 8.28 (d, 1H, H_{10}); 6.40 (m, 4H, 30H, NH^+); 4.30 (s, 2H, CH-CH), 3.08, 2.96, 2.40 and 2.02 (4s, 12H, $4CH_3$). Anal $C_{24}H_{24}N_2O_8$ (C, H, N).

4-Hydroxy-2,5,11-trimethyl-1H-6H-pyrido[3,2-b]carbazolium (oxalate) ***15a***
Prepared analogously to **9d**, yield: 30%; yellow solid, mp: > 270°C. IR: 3200 (NH); 3340 (OH); 2800, 2710 (COO^-). ^{1}H-NMR (DMSO–d_6) δ: 11.03 (s, H, NH); 5.90 (s, 1H; H3); 7.43, 7.10 (2m, 3H, $H_{7/8}$, H_9); 8.26 (d, 1H, H_{10}); 6.23 (m, 3H, 20H, NH^+); 3.06, 2.96, 2.46 (3s, 9H, $3CH_3$). Anal $C_{20}H_{18}N_2O_5$ (C, H, N).

5,11-Dimethyl-2-ethyl-4-hydroxy-1H-6H-pyrido[3,2-b]carbazolium (sulfate) ***15b***
Prepared analogously to **9d**, yield: 44%; yellow solid, mp: > 270°C. IR: 3180 (NH); 3380 (OH); 2760, 2600 (HSO_4^-). ^{1}H-NMR (DMSO–d_6) δ: 12.13, 11.02 (2s, 2H, OH, NH); 6.86 (s, 1H, H_3); 7.63, 7.26 (2m, 3H, $H_{7/8}$, H_9); 8.40 (d, 1H, H_{10}); 4.26 (s, 1H, NH^+); 3.20 (q, 2H, CH_2), 3.06, 3.00 (2s, 6H, $2CH_3$); 1.36 (t, 3H, CH_3). Anal $C_{19}H_{20}N_2O_5S$ (C, H, N).

L1210 cytotoxicity determination

Cell cultures and *in vitro* cytotoxicity determination were carried out following the procedures described previously [25]. Briefly, a 2-layer soft-agar culture was used for the clonogenic assay. The drugs, dissolved in DMSO, were diluted in RPMI 1640 and assayed in triplicate at each of the 3 following final concentrations: 0.1, 1 and 10 μg/ml. Two drug exposure protocols were used. In one case, the cells were incubated with drugs for 1 h, washed twice and then cloned in soft agar in multi-well plates. In the other, drugs were added directly in soft agar (continuous exposure). In both cases, cells were cloned at a final concentration of 40 000 cells per ml (12 000 cells/well). Colonies were counted after 5–7d of culture. We usually found 7200 colonies in the untreated wells, with a cloning efficiency of 60% ± 5%. The average number of colonies in each triplicate-treated culture was expressed as a percentage of the average number of colonies in the untreated controls. A compound was considered active if it reduced colony formation to 50% or less of the control value.

Acknowledgments

We thank J Robillard, Director of the Centre F Baclesse, and his successor JF Héron for their constant support. We are most grateful to M Aubert, C Boissart and MC Bon for technical assistance. This work was supported by grants from the Ligues Nationales et Départementales de Lutte Contre le Cancer (Calvados, Manche), the Association pour le Développement de la Recherche sur le Cancer (ARC) and the University of Caen.

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