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In vitro cytotoxicity of carbazole derivatives. V. 9-Halogeno-substituted 5,11-dimethyl-6*H*-pyrido[3,2-*b*]carbazoles

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Summary — Thirty-seven new 5,11-dimethyl-6*H*-pyrido[3,2-*b*]carbazole derivatives have been synthesized. These compounds are structurally related to the 5,11-dimethyl-6*H*-pyrido[4,3-*b*]carbazole antitumor drug ellipticine. They bear either a fluorine, a bromine or a chlorine atom on position 9, and are variously substituted on the pyridine ring. Twenty-nine of these compounds (78%) were found active when tested in vitro for their cytotoxic activity in a clonogenic assay using murine leukemia L1210 cell line. Structure–activity relationships are described in detail.

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

Introduction

A number of derivatives in the series of ellipticine, 5,11-dimethyl-6*H*-pyrido-[4,3-*b*]carbazoles, show interesting antitumor properties [1–3]. We have studied a series of structurally related compounds, 5,11-dimethyl-6*H*-pyrido[3,2-*b*]carbazoles, among which we have found cytotoxic molecules, as described in a previous paper [4]. In the present paper, we describe the synthesis of 9-halogeno derivatives, diversely substituted on C-2 and C-3, and bearing a hydroxyl (pyridocarbazolones) or an ethoxy group on C-4. We also report their cytotoxic activity against murine leukemia L1210 cells, determined by a clonogenic assay.

Chemistry

We describe here a convenient approach for the construction of 9-fluoro, 9-chloro or 9-bromo-5,11-dimethyl-6*H*-pyrido[3,2-*b*]carbazole derivatives making use of 6-halogeno-3-amino-1,4-dimethyl carbazoles **1–3** [5–7] (schemes 1 and 2). Compounds **1–3** were obtained in two steps starting from 6-halogeno-1,4-dimethyl carbazoles [8, 9], first by nitration in acetic anhydride, followed by reduction with stannous chloride in a mixture of dimethylformamide, hydrochloric acid and acetic acid. Condensation of various β-ketoesters with the amines **1–3** led to a non-isolable intermediary which was cyclized by heating at 200 °C in diphenylether to give the pyridocarbazolones **4a–l** and **6a–j** in 60% yield. These compounds exhibit lactam–lactim tautomerism [10, 11]. In a similar manner, condensation of ethyl propiolate with the amines **2** and **3** led to the corresponding pyridocarbazolones **8a** and **9a** in 50–60% yield. On the other hand, when diethylethoxymethylenemalonate was used, the reaction yielded the isolable intermediates **13** and **14** which gave the 4-ethoxypyridines **13a** and **14a** in 30% yield by sublimation in vacuo at 260 °C [4, 12]. The pyrido[3,2-*b*]carbazoles **6i**, **6j** and **4k** reacted in ethanol with a slight excess of hydrazine hydrate, providing the corresponding hydrazides **10–12**. Because some of these compounds were not very soluble, their salts **5**, **7**, **8b** and **9b** were prepared from oxalic or methane sulfonic acids.

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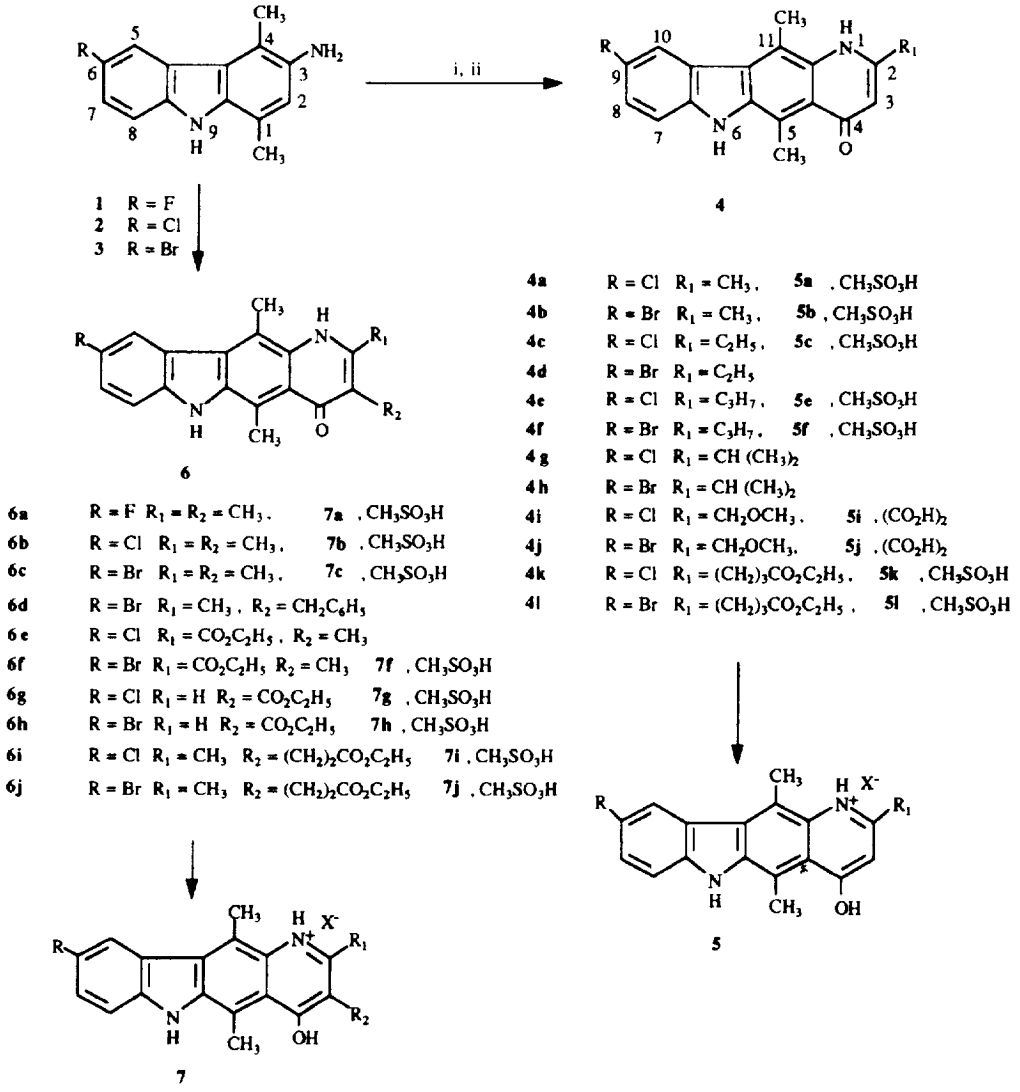


Scheme 1.

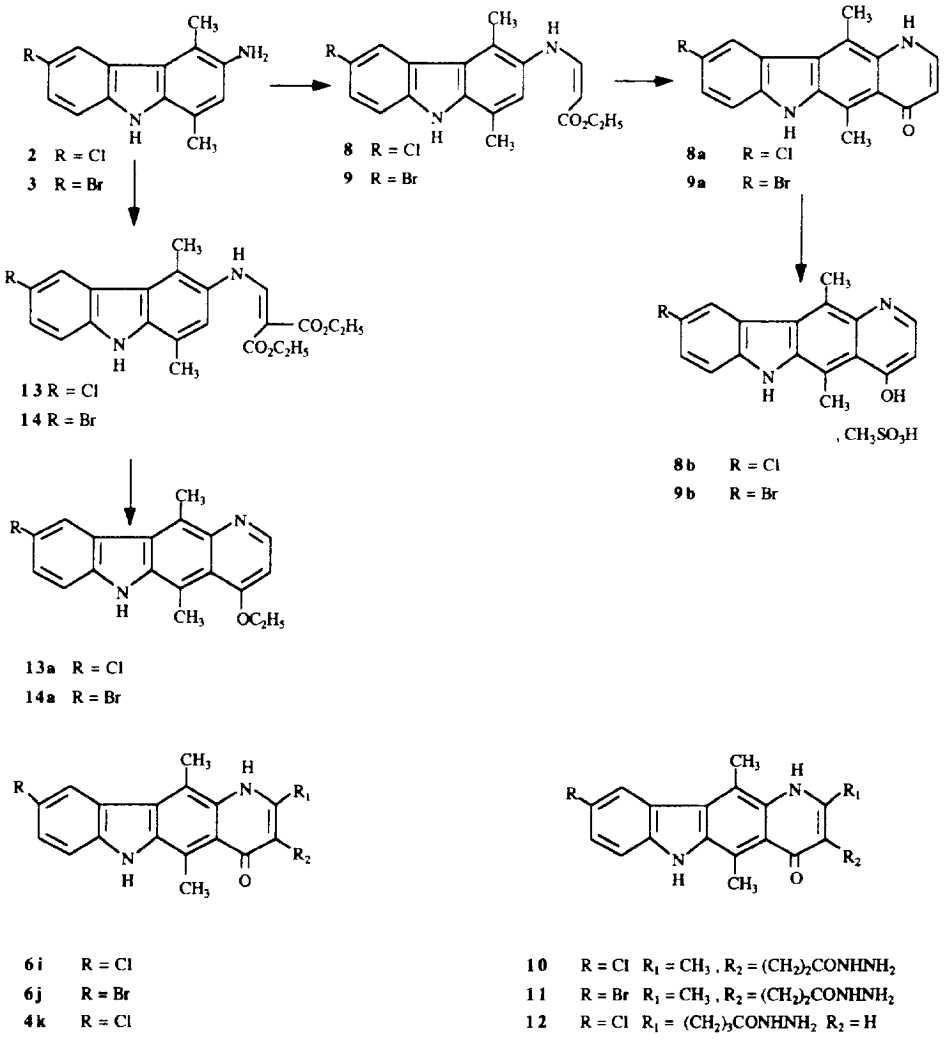
Biological results and discussion

Thirty-seven 5,11-dimethyl-6*H*-pyrido[3,2-*b*]carbazole derivatives, bearing a halogen atom on C-9, were evaluated in vitro against L1210 murine leukemia. The results are presented in table I. Twenty-nine derivatives were cytotoxic and most of them totally inhibited colony formation when assayed at 10 µg/mL using continuous exposure. Five 5,11-dimethyl-6*H*-pyrido[3,2-*b*]carbazoles, **4a**, **6g**, **7g**, **8b** and **9a** as well as the two compounds bearing an ethoxy group on C-4, **13a** and **14a**, were the most cytotoxic and totally inhibited colony formation at 10 µg/mL using brief exposure. Under the same conditions, nine other compounds (**4b**, **5a**, **5b**, **6a**, **6b**, **6c**, **7a**, **7b** and **7h**) inhibited colony formation by more than 80%. Compounds **9a**, **13a** and **14a** were as active as the reference compound *N*2-methyl-9-hydroxyellipticinium acetate (NMHE).

From the structure–activity point of view, some features can be pointed out.

Role of the pyridine ring

Contrary to what we have previously observed for C-9 non-substituted analogues [4], the 9-halogeno-5,11-dimethyl-6*H*-pyrido[3,2-*b*]carbazole derivatives are more active than their tricyclic precursors, 3-amino-6-halogeno-1,4-dimethylcarbazoles (**2/8b** and **3/9a**).



Scheme 2.

Influence of halogenation at C-9

Comparison of the present results with data presented in reference [4] show that for 5,11-dimethyl-6*H*-pyrido[3,2-*b*]carbazole derivatives, introduction of a fluorine (**6a**), chlorine (**6b**, **13a**) or bromine atom (**6c**, **14a**) at C-9 is favorable to activity. These results contrast with those obtained with 9-chloro-, 9-bromo- and 9-fluoro ellipticine [13, 14] and with tricyclic analogues [9].

Influence of methylation on the pyridine ring

The C-2 and C-3 non-substituted derivatives are the most cytotoxic either in the 9-bromo- (**9a**) or the 9-chloro series (**8a**). However, in the latter case, the base (**8a**) appears less active than the methanesulfonate (**8b**), probably as a consequence of its lower water solubility. Introduction of a methyl group at C-2 decreases the cytotoxicity of the 9-halogeno derivatives (**5a/8b** and **4b/9a**), whereas addition of a second methyl group on C-3 does not notably change their activity (**7b/5a** and **6c/4b**). Thus, leaving aside solubility problems, the absence of methyl groups on the pyridine ring seems favorable to activity, although methylated compounds retain notable activity.

Influence of an alkyl chain on C-2

For 9-halogeno-5,11-dimethyl-6*H*-pyrido[3,2-*b*]carbazoles non-substituted on C-3 and bearing a 2-alkyl side chain, the cytotoxicity decreases as the number of carbon units in the side chain increases (**4c** to **4h**).

$$R_1: H > CH_3 > C_2H_5 > CH(CH_3)_2 > (CH_2)_2CH_3$$

Table I. In vitro cytotoxicity of 5,11-dimethyl-6*H*-pyrido[3,2-*b*]carbazoles.

Compound	*Colony formation (% of control)*						*Activity*	
	Continuous exposure (CE)			*One hour exposure (BE)*			*CE*	*BE*
	10 μg/mL	*1 μg/mL*	*0.1 μg/mL*	*10 μg/mL*	*1 μg/mL*	*0.1 μg/mL*		
NMHE	0 ± 0	28 ± 2	85 ± 3	0 ± 0	78 ± 2	98 ± 3	+++	++
1*	82 ± 3	100 ± 3	101 ± 1	98 ± 2	97 ± 2	99 ± 2	–	–
2*	51 ± 3	79 ± 4	93 ± 2	96 ± 2	98 ± 3	99 ± 1	±	–
3*	7 ± 2	55 ± 4	81 ± 2	40 ± 3	60 ± 2	97 ± 2	+	±
4a	0 ± 0	74 ± 2	98 ± 3	0 ± 0	83 ± 5	100 ± 3	++	++
4b	0 ± 0	79 ± 5	93 ± 5	10 ± 2	88 ± 5	99 ± 5	++	+
4c	NT	NT	NT	NT	NT	NT	NT	NT
4d	NT	NT	NT	NT	NT	NT	NT	NT
4e	76 ± 3	83 ± 2	97 ± 3	91 ± 2	95 ± 2	97 ± 2	–	–
4f	NT	NT	NT	NT	NT	NT	NT	NT
4g	76 ± 3	83 ±2	97 ± 2	91 ± 2	96 ± 2	97 ± 2	–	–
4h	36 ± 1	85 ± 2	97 ± 3	68 ± 2	87 ± 3	97 ± 2	±	–
4i	0 ± 0	85 ± 4	96 ± 3	45 ± 6	87 ± 4	98 ± 3	++	±
4j	0 ± 0	73 ± 4	87 ± 4	77 ± 3	86 ± 4	98 ± 3	++	–
4k	NT	NT	NT	NT	NT	NT	NT	NT
4l	46 ± 2	89 ± 3	94 ± 1	88 ± 3	94 ± 2	100±1	±	–
5a	0 ± 0	79 ± 3	99 ± 3	20 ± 1	85 ± 2	99 ± 3	++	+
5b	0 ± 0	85 ± 2	99 ± 2	14 ± 2	97 ± 2	99 ± 3	++	+
5c	0 ± 0	79 ± 2	94 ± 4	45 ± 3	81 ± 2	97 ± 3	++	±
5d	58 ± 2	71 ± 3	94 ± 2	75 ± 3	89 ± 3	98 ± 3	–	–
5f	54 ± 2	90 ± 4	97 ± 6	75 ± 6	94 ± 4	98 ± 3	–	–
5i	0 ± 0	72 ± 4	84 ± 4	48 ± 4	89 ± 3	97 ± 3	++	±
5j	0 ± 0	77 ± 2	100 ± 2	41 ± 2	94 ± 2	96 ± 2	++	±
5k	34 ± 2	66 ± 3	97 ± 2	70 ± 2	91 ± 2	99 ± 2	±	–
5l	67 ± 3	87 ± 3	91 ± 1	85 ± 3	96 ± 2	99 ± 1	–	–
6a	0 ± 0	91 ± 3	99 ± 2	4 ± 0	95 ± 2	99 ± 1	++	+
6b	0 ± 0	72 ± 2	99 ± 2	3 ± 1	86 ± 3	97 ± 2	++	+
6c	0 ± 0	81 ± 3	98 ± 3	21 ± 2	93 ± 4	98 ± 3	++	+
6d	NT	NT	NT	NT	NT	NT	NT	NT
6e	NT	NT	NT	NT	NT	NT	NT	NT
6f	NT	NT	NT	NT	NT	NT	NT	NT
6g	0 ± 0	89 ± 4	96 ± 3	0 ± 0	96 ± 2	100 ± 3	++	++
6h	NT	NT	NT	NT	NT	NT	NT	NT
6i	NT	NT	NT	NT	NT	NT	NT	NT
6j	0 ± 0	92 ± 2	98 ± 2	82 ± 2	97 ± 2	97 ± 2	++	–
7a	0 ± 0	90 ± 2	100 ± 3	20 ± 2	99 ± 2	100 ± 2	++	+
7b	0 ± 0	74 ± 0	96 ± 3	3 ± 1	99 ± 2	100 ± 2	++	+
7c	NT	NT	NT	NT	NT	NT	NT	NT
7f	NT	NT	NT	NT	NT	NT	NT	NT
7g	0 ± 0	76 ± 4	99 ± 2	0 ± 0	88 ± 1	98 ± 3	++	++
7h	0 ± 0	75 ± 2	97 ± 4	10 ± 1	89 ± 3	99 ± 1	++	+
7i	0 ± 0	59 ± 1	94 ± 2	64 ± 3	94 ± 2	98 ± 3	++	–
7j	80 ± 3	92 ± 3	98 ± 4	95 ± 1	98 ± 3	98 ± 2	–	–
8a	12 ± 2	51 ± 2	81 ± 1	59 ± 3	81 ± 2	95 ± 2	+	–
9a	0 ± 0	28 ± 2	69 ± 4	0 ± 0	62 ± 2	93 ± 3	+++	++
8b	0 ± 0	87 ± 8	96 ± 5	0 ± 0	80 ± 5	103 ± 4	++	++
9b	NT	NT	NT	NT	NT	NT	NT	NT
10	91 ± 2	97 ± 3	99 ± 3	94 ± 2	97 ± 1	99 ± 3	–	–
11	NT	NT	NT	NT	NT	NT	NT	NT
12	78 ± 2	87 ± 2	97 ± 2	86 ± 2	93 ± 3	97 ± 2	–	–
13a	0 ± 0	0 ± 0	72 ± 1	0 ± 0	58 ± 4	82 ± 2	+++	++
14a	0 ± 0	0 ± 0	76 ± 3	0 ± 0	72 ± 3	90 ± 2	+++	++

NT: not tested; * results published in reference [9].

This behavior has also been observed with the C-9-non-substituted analogues [4], and suggests that bulkiness of the chain modulates the activity. Meanwhile, replacing the 2-alkyl chain by the 2-methoxymethyl chain of equivalent bulkiness increases the cytotoxicity of both 9-chloro and 9-bromo derivatives (**4i/4g** and **4j/4h**). This was not observed with the C-9 non-substituted analogues [4]. The former observation suggests that parameters such as hydro- and liposolubility also influence the activity of the derivatives, while the latter suggests that the mechanism(s) of action might be somehow different for C-9 non-substituted and 9-halogeno derivatives. For the 9-halogeno 5,11-dimethyl-6*H*-pyrido[3,2-*b*]carbazoles, addition of an ethoxycarbonyl (**5k**) or $CONHNH_2$ group (**12**) to a propyl group at C-2 strongly inhibits the activity.

Influence of substitution at C-3

Addition of ethoxycarbonyl group on C3 leads to very active compounds (**7g** and **7h**). Addition of this group (**7i** and **7j**) or a $CONHNH_2$ group (**10** and **11**) to an ethyl or a propyl group at C-3 leads to less active or inactive derivatives:

R_2: $CO_2C_2H_5 > CH_3 > (CH_2)_2CO_2C_2H_5 > (CH_2)_3CONHNH_2$

Influence of substitution at C-4

The two derivatives bearing an ethoxy group on C-4 (**13** and **14**) are the only compounds of the present series that totally inhibit the colony formation at 1 μg/mL under continuous exposure. They bear no methyl group(s) on the pyridine ring, like the very active derivative **9a**, a result which confirms that methyl groups are not necessary for activity. The C-9 non-substituted 4-ethoxy analogue was also very active, but only partially inhibited cell proliferation at 1 μg/mL [4].

Conclusion

The results presented in this paper confirm the interest of the 5,11-dimethyl-6*H*-pyrido[3,2-*b*]carbazole series, twenty-nine new derivatives being cytotoxic in L1210 leukemia cell culture. The presence of a halogen atom on C-9 appears favorable to activity, whether or not the derivatives are methylated on the pyridine ring. Some of these 5,11-dimethyl-6*H*-pyrido[3,2-*b*]carbazoles have been tested in vivo for their antineoplastic activity against murine tumors, and gave promising results. Considering that 9-chloroellipticine has recently been reported to show anticancer specificity against human brain tumors in vitro and in vivo [14, 15], it appears from our results that 5,11-dimethyl-6*H*-pyridocarbazoles bearing a halogen atom on C-9 may be an interesting route to the development of new antitumoral drugs.

Experimental protocols

Chemistry

10β-Ketoesters (ethyl acetoacetate, ethyl propionylacetate, ethyl butyrylacetate, ethyl isobutyrylacetate, 2-methoxyethyl acetoacetate, diethyl-3-oxopimelate, ethyl-2-methyl acetoacetate, ethyl-2-benzyl acetoacetate, diethyl oxalpropionate, diethyl-2-acetyl glutarate, diethyl ethoxy methylene malonate) were obtained from Aldrich (France).

Melting points were determined on a Kofler type NME 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 **1–3**, **13**, **14**, **13a** and **14a** have been described in a previous paper [16]. Physical data for compounds **4b–l** and **6a–j** are given in table II and for compounds **5b–l**, **7a–j**, **8b** and **9b** in table III.

9-Chloro-4-hydroxy-2,5,11-trimethyl-6H-pyrido[3,2-b]carbazole ***4a****. General procedure*

A mixture of 3-amino-6-chloro-1,4-dimethyl-9*H*-carbazole **2** (10 g, 0.0408 mol) and ethylacetoacetate (10.61 g, 0.0816 mol), acetic acid 2 mL in benzene 130 mL was refluxed under nitrogen atmosphere for 1 h. The solvent was then evaporated in vacuo and the oil product was dissolved in 50 mL of diphenyl ether and was heated at 200 °C for 10 min. The solid product was filtered and crystallized from acetonitrile to give **4a**, yield (8.10 g , 64%), as yellow crystals, mp > 270 °C. IR (KBr): ν 3200 (NH), 3450 (OH) cm^{-1}. ^{1}H-NMR (DMSO-d_6) δ: 11.09 (s, 1H, NH), 9.86 (s, 1H, OH), 8.16 (d, 1H, H-10), 7.40 (m, 2H, H-7,8), 5.70 (s, 1H, H-3), 3.10, 2.93, 2.36 (s, 9H, CH_3 x 3). Anal $C_{18}H_{15}ClN_2O$ (C, H, N, Cl).

The yields and conditions for the isolated products **4a–l** and **6a–j** are summarized in the table II.

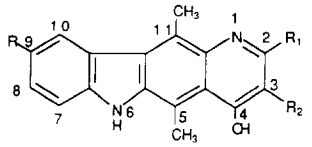
9-Chloro-4-hydroxy-2,5,11-trimethyl-1H$^+$-6H-pyrido[3,2-b]-carbazolium methane sulfonate ***5a****. General procedure*

A mixture of **4a** (5 g, 0.0161 mol), methane sulfonic acid (2.31 g, 0.0241 mol) and ethanol (350 mL) in DMSO (30 mL) was heated under nitrogen at 60 °C for 2 h. The resulting crystals were collected, washed with ethanol, dried and recrystallized from acetonitrile to yield (5.8 g, 88%), mp > 280 °C. IR (KBr): ν 3440 (OH), 3200 (NH), 1170 (SO_3^-) cm^{-1}; ^{1}H-NMR (DMSO-d_6) δ: 11.35 (s, 1H, NH), 5.80 (s, 2H, OH, NH+), 7.90 (d, 1H, H10), 7.42 (dd, 2H, H8,7), 6.70 (s, 1H, H3), 2.85, 2.75 (s, 12H, CH_3 x 4). Anal $C_{19}H_{18}ClN_2O_4S$ (C, H, Cl, N, S).

9-Chloro-5,11-dimethyl-4-hydroxy-2-methoxymethyl-1H$^+$-6H-pyrido-[3,2-b]carbazolium oxalate ***5i***

A solution of **4i** (3 g, 0.0088 mol) and oxalic acid (0.95 g, 0.0105 mol) in isopropanol (120 mL) was refluxed under nitrogen atmosphere for 1 h. The resulting precipitates were collected and recrystallized from acetonitrile to afford (2.6 g,

Table II. Physical data for 5,11-dimethyl-6*H*-pyrido[3, 2-*b*]carbazoles **4b–l** and **6a–j**.



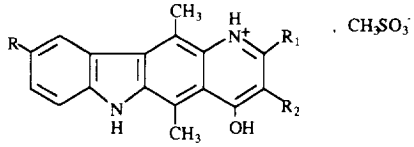
Compound	*R*	R_1	R_2	*Mp*[a,b] (°C)	*Yield (%)*	*Formula*	*^{1}H-NMR (δ, ppm)*[c]
4b	Br	CH_3	H	267[a]	60	$C_{18}H_{15}BrN_2O$	11.33 (s, 1H, NH), 9.93 (s, 1H, OH), 8.40 (d, 1H, H10), 7.50 (dd, 2H, H8,7), 5.76 (s, 1H, H3), 3.06, 2.91, 2.40 (s, 9H, CH_3 x 3)
4c	Cl	C_2H_5	H	278[a]	56	$C_{19}H_{17}ClN_2O$	11.40 (s, 1H, NH), 10.15 (s, 1H, OH), 8.23 (d, 1H, H10), 7.40 (dd, 2H, H8,7), 5.70 (s, 1H, H3), 3.00, 2.85 (s, 6H, CH_3 x 2), 2.60 (q, 2H, CH_2), 1.20 (t, 3H, CH_3)
4d	Br	C_2H_5	H	270[a]	58	$C_{19}H_{17}BrN_2O$	11.35 (s, 1H, NH), 9.90 (s, 1H, OH), 8.30 (d, 1H, H10), 7.42 (dd, 2H, H8,7), 5.72 (s, 1H, H3), 3.04, 2.82 (s, 6H, CH_3 x 2), 2.62 (q, 2H, CH_2), 1.24 (t, 3H, CH_3)
4e	Cl	C_3H_7	H	>280[b]	29	$C_{20}H_{19}ClN_2O$	11.15 (s, 1H, NH), 9.75 (s, 1H, OH), 8.15 (d, 1H, H10), 7.35 (dd, 2H, H8,7), 5.70 (s, 1H, H3), 3.00, 2.90 (s, 6H, CH_3 x 2), 2.60, 1.62 (m, 4H, CH_2 x 2), 0.90 (m, 3H, CH_3)
4f	Br	C_3H_7	H	>280[b]	56	$C_{20}H_{19}BrN_2O$	11.35 (s, 1H, NH), 9.30 (s, 1H, OH), 8.32 (d, 1H, H10), 7.40 (dd, 2H, H8,7), 5.75 (s, 1H, H3), 3.00, 2.92 (s, 6H, CH_3 x 2), 2.64, 1.60 (m, 4H, CH_2 x 2), 0.95 (m, 3H, CH_3)
4g	Cl	$CH(CH_3)_2$	H	>280[b]	29	$C_{20}H_{19}ClN_2O$	11.15 (s, 1H, NH), 9.30 (s, 1H, OH), 8.27 (d, 1H, H10), 7.45 (dd, 2H, H8,7), 5.80 (s, 1H, H3), 3.30 (m, 1H, CH), 3.05, 2.90 (s, 6H, CH_3 x 2), 1.25, 1.15 (s, 6H, CH_3 x 2)
4h	Br	$CH(CH_3)_2$	H	>280[b]	30	$C_{20}H_{19}BrN_2O$	11.20 (s, 1H, NH), 9.27 (s, 1H, OH), 8.27 (d, 1H, H10), 7.45 (dd, 2H, H8,7), 5.80 (s, 1H, H3), 3.28 (m, 1H, CH), 3.05, 2.90 (s, 6H, CH_3 x 2), 1.25, 1.15 (s, 6H, CH_3 x 2)
4i	Cl	CH_2OCH_3	H	>280[b]	53	$C_{19}H_{17}ClN_2O$	11.09 (s, 1H, NH), 9.83 (s, 1H, OH), 8.13 (d, 1H, H10), 7.33 (dd, 2H, H8,7), 5.83 (s, 1H, H3), 4.40 (s, 2H, CH_2), 3.33 (s, 3H, CH_3), 3.00, 2.83 (s, 6H, CH_3 x 2)
4j	Br	CH_2OCH_3	H	>280[b]	55	$C_{19}H_{17}BrN_2O$	11.06 (s, 1H, NH), 9.76 (s, 1H, OH), 8.26 (d, 1H, H10), 7.40 (dd, 2H, H8,7), 5.83 (s, 1H, H3), 4.42 (s, 2H, CH_2), 3.30 (s, 3H, CH_3), 3.00, 2.83 (s, 6H, CH_3 x 2)
4k	Cl	$(CH_2)_3CO_2C_2H_5$	H	>280[b]	26	$C_{23}H_{23}ClN_2O_3$	11.15 (s, 1H, NH), 9.85 (s, 1H, OH), 8.15 (d, 1H, H10), 7.40 (dd, 2H, H8,7), 5.70 (s, 1H, H3), 4.03 (q, 2H, CH_2), 3.05, 2.92 (s, 6H, CH_3 x 2), 2.60, 2.35, 1.95 (m, 6H, CH_2 x 3), 1.20 (t, 3H, CH_3)

Table II. *Continued.*

Compound	*R*	R_1	R_2	*Mp*[a,b] (°C)	*Yield (%)*	*Formula*	*^{1}H-NMR (δ, ppm)*[c]
4l	Br	$(CH_2)_3CO_2C_2H_5$	H	>280[b]	28	$C_{23}H_{23}BrN_2O_3$	11.20 (s, 1H, NH), 9.92 (s, 1H, OH), 8.35 (d, 1H, H10), 7.50 (dd, 2H, H8,7), 5.75 (s, 1H, H3), 4.19 (q, 2H, CH_2), 3.09, 2.97 (s, 6H, CH_3 x 2), 2.70, 2.40, 2.00 (m, 6H, CH_2 x 3), 1.20 (t, 3H, CH_3)
6a	F	CH_3	CH_3	267[a]	44	$C_{19}H_{17}FN_2O$	10.86 (s, 1H, NH), 9.56 (s, 1H, OH), 7.90 (dd, 1H, H10), 7.33, 7.18 (m, 2H, H8,7), 3.20, 2.96, 2.76, 1.80 (s, 12H, CH_3 x 4)
6b	Cl	CH_3	CH_3	>280[b]	60	$C_{19}H_{17}ClN_2O$	11.10 (s, 1H, NH), 9.70 (s, 1H, OH), 8.16 (d, 1H, H10), 7.43 (dd, 2H, H8,7), 3.10, 2.90, 2.43, 1.93 (s, 12H, CH_3 x 4)
6c	Br	CH_3	CH_3	>280[b]	60	$C_{19}H_{17}BrN_2O$	11.13 (s, 1H, NH), 9.73 (s, 1H, OH), 8.33 (d, 1H, H10), 7.46 (dd, 2H, H8,7), 3.10, 3.00, 2.43, 1.93 (s, 12H, CH_3 x 4)
6d	Br	CH_3	$CH_2C_6H_5$	>280[b]	54	$C_{25}H_{25}BrN_2O$	11.15 (s, 1H, NH), 9.80 (s, 1H, OH), 8.37 (d, 1H, H10), 7.50 (dd, 2H, H8,7), 7.18 (m, 5H, C_6H_5), 3.95 (s, 2H, CH_2), 3.17, 3.00, 2.50 (s, 9H, CH_3 x 3)
6e	Cl	$CO_2C_2H_5$	CH_3	270[a]	28	$C_{21}H_{19}ClN_2O_3$	11.20 (s, 1H, NH), 9.60 (s, 1H, OH), 8.35 (d, 1H, H10), 7.40 (dd, 2H, H8,7), 4.08 (q, 2H, CH_2), 3.20, 3.00, 2.48 (s, 9H, CH_3 x 3), 1.20 (t, 3H, CH_3)
6f	Br	$CO_2C_2H_5$	CH_3	>280[b]	27	$C_{21}H_{19}BrN_2O_3$	11.30 (s, 1H, NH), 9.80 (s, 1H, OH), 8.30 (d, 1H, H10), 7.42 (dd, 2H, H8,7), 4.10 (q, 2H, CH_2), 3.17, 3.00, 2.46 (s, 9H, CH_3 x 3), 1.22 (t, 3H, CH_3)
6g	Cl	H	$CO_2C_2H_5$	>280[b]	44	$C_{20}H_{17}ClN_2O_3$	11.32 (s, 2H, NH, OH), 8.25 (d, 1H, H10), 7.43 (dd, 2H, H8,7), 8.30 (s, 1H, H2), 4.20 (q, 2H, CH_2), 3.05, 2.95 (s, 6H, CH_3 x 2), 1.30 (t, 3H, CH_3)
6h	Br	H	$CO_2C_2H_5$	>280[b]	43	$C_{20}H_{17}BrN_2O_3$	11.35 (s, 2H, NH, OH), 8.24 (d, 1H, H10), 7.45 (dd, 2H, H8,7), 8.30 (s, 1H, H2), 4.20 (q, 2H, CH_2), 3.10, 2.96 (s, 6H, CH_3 x 2), 1.30 (t, 3H, CH_3)
6i	Cl	CH_3	$(CH_2)_2CO_2C_2H_5$	>280[b]	67	$C_{23}H_{23}ClN_2O_3$	10.95 (s, 1H, NH), 9.50 (s, 1H, OH), 8.00 (d, 1H, H10), 7.30 (dd, 2H, H8,7), 3.95 (q, 2H, CH_2), 2.93, 2.75, 2.34 (s, 9H, CH_3 x 3), 2.30 (m, 4H, CH_2 x 2), 1.13 (t, 3H, CH_2)
6j	Br	CH_3	$(CH_2)_2CO_2C_2H_5$	>280[b]	65	$C_{23}H_{23}BrN_2O_3$	11.10 (s, 1H, NH), 9.68 (s, 1H, OH), 8.27 (d, 1H, H10), 7.40 (dd, 2H, H8,7), 4.00 (q, 2H, CH_2), 3.03, 2.85, 2.45 (s, 9H, CH_3 x 3), 2.33 (m, 4H, CH_2 x 2), 1.18 (t, 3H, CH_3)

[a,b]Crystallization solvents; a = acetonitrile, b = dimethylformamide. [c]Chemical shifts (DMSO-d_6/TMS) δ in ppm. **6a** $J_{10\text{-}F}$ = 9.90 Hz, $J_{10\text{-}8}$ = 2.40 Hz, $J_{8\text{-}F}$ = 9.90 Hz, $J_{8\text{-}7}$ = 9.00 Hz, $J_{7\text{-}F}$ = 4.80 Hz. **6b–i** $J_{10\text{-}8}$ = 1.80 Hz, $J_{8\text{-}7}$ = 8.40 Hz.

Table III. Physical data for 5,11-dimethyl-6*H*-pyrido[3,2-*b*]carbazole salts **5b–l**, **7a–j**, **8b** and **9b**.



Compound	*R*	*R_1*	*R_2*	*Mp (°C)*	*Yield (%)*	*Formula*	*^{1}H-NMR (δ, ppm)*
5b	Br	CH_3	H	270	49	$C_{19}H_{19}BrN_2O_4S$	11.40 (s, 1H, NH), 6.00 (s, 1H, OH), 5.60 (s, 1H, NH+), 7.96 (d, 1H, H10), 7.40 (dd, 2H, H8,7), 6.70 (s, 1H, H3), 2.80 (s, 3H, CH_3), 2.72 (s, 9H, CH_3 x 3)
5c	Cl	C_2H_5	H	>280	34	$C_{20}H_{21}ClN_2O_4S$	11.35 (s, 1H, NH), 4.70 (s, 1H, OH), 4.80 (s, 1H, NH+), 8.07 (d, 1H, H10), 7.43 (dd, 2H, H8,7), 6.78 (s, 1H, H3), 3.17 (q, 2H, CH_2), 3.00 (s, 3H, CH_3), 2.45 (s, 6H, CH_3 x 2), 1.35 (t, 3H, CH_3)
5e	Cl	C_3H_7	H	>280	39	$C_{21}H_{23}ClN_2O_4S$	11.40 (s, 1H, NH), 4.30 (s, 1H, OH), 4.35 (s, 1H, NH+), 8.10 (d, 1H, H10), 7.45 (dd, 2H, H8,7), 6.82 (s, 1H, H3), 3.00 (s, 3H, CH_3), 2.60 (s, 6H, CH_3 x 2), 1.85 (m, 4H, CH_2 x 2), 1.15 (t, 3H, CH_3)
5f	Br	C_3H_7	H	>280	38	$C_{21}H_{23}BrN_2O_4S$	11.38 (s, 1H, NH), 4.39 (s, 1H, OH), 4.30 (s, 1H, NH+), 8.00 (d, 1H, H10), 7.45 (dd, 2H, H8,7), 6.82 (s, 1H, H3), 3.00 (s, 3H, CH_3), 2.65 (s, 6H, CH_3 x 2), 1.85 (m, 4H, CH_2 x 2), 1.15 (t, 3H, CH_3)
5k	Cl	$(CH_2)_3CO_2C_2H_5$	H	>280	36	$C_{24}H_{27}ClN_2O_6S$	11.30 (s, 1H, NH), 5.20 (m, 2H, OH, NH+), 7.94 (d, 1H, H10), 7.38 (dd, 2H, H8,7), 6.75 (s, 1H, H3), 4.00, 2.00 (m, 8H, CH_2 x 4), 2.93 (s, 3H, CH_3), 2.47 (s, 6H, CH_3 x 2), 1.17 (t, 3H, CH_3)
5l	Br	$(CH_2)_3CO_2C_2H_5$	H	>280	37	$C_{24}H_{27}BrN_2O_6S$	11.27 (s, 1H, NH), 6.00 (m, 2H, OH, NH+), 8.05 (d, 1H, H10), 7.37 (dd, 2H, H8,7), 6.73 (s, 1H, H3), 4.20, 2.00 (m, 8H, CH_2 x 4), 2.85 (s, 3H, CH_3), 2.43 (s, 6H, CH_3 x 2), 1.18 (t, 3H, CH_3)
7a	F	CH_3	CH_3	>280	57	$C_{20}H_{21}FN_2O_4S$	11.10 (s, 1H, NH), 4.20 (m, 2H, OH, NH+), 7.95 (dd, 1H, H10), 7.32 (m, 2H, H8,7), 2.90, 2.70 (s, 6H, CH_3 x 2), 2.45 (s, 6H, CH_3 x 2), 2.30 (s, 3H, CH_3)
7b	Cl	CH_3	CH_3	>280	61	$C_{20}H_{21}ClN_2O_4S$	11.17 (s, 1H, NH), 4.20 (m, 2H, OH, NH+), 7.98 (d, 1H, H10), 7.35 (dd, 2H, H8,7), 2.90, 2.85, 2.65, 2.48, 2.25 (s, 15H, CH_3 x 5)
7c	Br	CH_3	CH_3	>280	58	$C_{20}H_{21}BrN_2O_4S$	11.18 (s, 1H, NH), 4.25 (m, 2H, OH, NH+), 8.05 (d, 1H, H10), 7.38 (dd, 2H, H8,7), 2.95, 2.70 (s, 6H, CH_3 x 2), 2.50 (s, 6H, CH_3 x 2), 2.30 (s, 3H, CH_3)
7f	Br	$CO_2C_2H_5$	CH_3	>280	24	$C_{22}H_{23}BrN_2O_6S$	10.90 (s, 1H, NH), 5.83 (m, 2H, OH, NH+), 7.95 (d, 1H, H10), 7.15 (dd, 2H, H8,7), 4.19 (q, 2H, CH_2), 2.65, 2.58, 2.20, 1.80 (s, 12H, CH_3 x 4), 1.10 (t, 3H, CH_3)

Table III. *Continued.*

Compound	*R*	R_1	R_2	*Mp (°C)*	*Yield (%)*	*Formula*	*^{1}H-NMR (δ, ppm)*
7g	Cl	H	$CO_2C_2H_5$	>280	32	$C_{21}H_{21}ClN_2O_6S$	11.12 (s, 1H, NH), 5.32 (m, 2H, OH, NH+), 8.10 (s, 1H, H2), 7.80 (d, 1H, H10), 7.28 (dd, 2H, H8,7), 4.20 (q, 2H, CH_2), 2.70, 2.55, 2.50 (s, 9H, CH_3 x 3), 1.33 (t, 3H, CH_3)
7h	Br	H	$CO_2C_2H_5$	>280	36	$C_{21}H_{21}BrN_2O_6S$	11.05 (s, 1H, NH), 6.15 (m, 2H, OH, NH+), 7.97 (s, 1H, H2), 7.78 (d, 1H, H10), 7.18 (dd, 2H, H8,7), 4.10 (q, 2H, CH_2), 2.65, 2.47, 3.35 (s, 9H, CH_3 x 3), 1.20 (t, 3H, CH_3)
7i	Cl	CH_3	$(CH_2)_2CO_2C_2H_5$	>280	37	$C_{24}H_{27}ClN_2O_6S$	11.07 (s, 1H, NH), 9.66 (m, 2H, OH, NH+), 8.23 (d, 1H, H10), 7.36 (dd, 2H, H8,7), 3.92 (q, 2H, CH_2), 2.93, 2.80 (s, 6H, CH_3 x 2), 2.40 (s, 6H, CH_3 x 2), 2.35 (m, 4H, CH_2 x 2), 1.10 (t, 3H, CH_3)
7j	Br	CH_3	$(CH_2)_2CO_2C_2H_5$	>280	40	$C_{24}H_{27}BrN_2O_6S$	11.10 (s, 1H, NH), 9.10 (m, 2H, OH, NH+), 8.20 (d, 1H, H10), 7.40 (dd, 2H, H8,7), 3.98 (q, 2H, CH_2), 2.90, 2.85 (s, 6H, CH_3 x 2), 2.48 (s, 6H, CH_3 x 2), 2.32 (m, 4H, CH_2 x 2), 1.12 (t, 3H, CH_3)
8b	Cl	H	H	>280	30	$C_{18}H_{17}ClN_2O_4S$	11.30 (s, 1H, NH), 4.45 (m, 2H, OH, NH+), 8.50 (d, 1H, H2), 6.90 (d, 1H, H3), 7.98 (d, 1H, H10), 7.35 (dd, 2H, H8,7), 2.85 (s, 3H, CH_3), 2.40 (s, 6H, CH_3 x 2)
9b	Br	H	H	>280	32	$C_{18}H_{17}BrN_2O_4S$	11.30 (s, 1H, NH), 4.35 (m, 2H, OH, NH+), 8.50 (d, 1H, H2), 6.95 (d, 1H, H3), 8.10 (d, 1H, H10), 7.45 (dd, 2H, H8,7), 2.85 (s, 3H, CH_3), 2.55 (s, 6H, CH_3 x 2)

67%) of **5i**, mp > 280 °C. IR (KBr): ν 3340 (OH), 3280 (NH), 2800, 2500, 1750 (NH+, COOH); cm^{-1}; ^{1}H-NMR (DMSO-d_6) δ: 11.12 (s, 1H, NH), 6.03 (s, 1H, OH), 6.03 (s, 1H, OH, NH+), 8.16 (d, 1H, H10), 7.36 (dd, 2H, H8,7), 5.76 (s, 1H, H3), 4.40 (s, 2H, CH_3), 3.40 (s, 3H, CH_3), 2.96, 2.86 (s, 6H, CH_3 x 2). Anal $C_{21}H_{19}ClN_2O_6$ (C, H, Cl, N).

9-Bromo-5,11-dimethyl-4-hydroxy-2-methoxymethyl-1H+-6H-pyrido[3,2-b]carbazolium oxalate ***5j***

Prepared analogously to **5i**, yield : (2.9 g, 78%), mp > 280 °C (acetonitrile). IR (KBr): ν 3200 (NH), 3350 (OH), 2800, 2500, 1750 (NH+, COOH); cm^{-1}; ^{1}H-NMR (DMSO-d_6) δ: 11.20 (s, 1H, NH), 6.50 (s, 1H, OH), 6.50 (s, 2H, NH+, OH), 8.30 (d, 1H, H10), 7.43 (dd, 2H, H8,7), 6.03 (s, 1H, H3), 4.43 (s, 2H, CH_2), 3.33 (s, 3H, CH_3), 3.00, 2.86 (s, 6H, CH_3 x 2). Anal $C_{21}H_{19}BrN_2O_6$ (C, H, Br, N).

Ethyl-3-(6-chloro-1,4-dimethyl-3-9H-carbazolylamino)acrylate ***8****. General procedure*

A solution of 5 g (0.0204 mol) of 3-amino-6-chloro-1,4-dimethyl-9*H*-carbazole **2** and 2 g (0.0204 mol) of ethyl propiolate in 50 mL ethanol was refluxed under nitrogen atmosphere for 1 h. The solid product was filtered and crystallized from acetonitrile to give **8**, yield (5.20 g, 74%), as yellow crystals, mp 222 °C. IR (KBr): ν 3300 (NH), 1630 (CO) cm^{-1}; ^{1}H-NMR (DMSO-d_6) δ: 11.17 (s, 1H, NH), 10.00 (d, 1H, NH-CH, $J_{NH,CH}$ = 12.60 Hz), 7.95 (d, 1H, H5), 7.25, 7.45 (m, 4H, H8-7-2-2'), 4.70 (d, 1H, H3', $J_{H3'-2'}$ = 7.80 Hz), 4.10 (q, 2H, CH_2), 2.60, 2.45 (s, 6H, CH_3 x 2), 1.25 (t, 3H, CH_3). Anal $C_{19}H_{19}ClN_2O_2$ (C, H, N).

Ethyl-3-(6-bromo-1,4-dimethyl-3-9H-carbazolylamino)acrylate ***9***

This compound was prepared with the general procedure described for **8**, yield (4.5 g, 67%), mp 220 °C (acetonitrile) as yellow crystals. IR (KBr): ν 3300 (NH), 1630 (CO) cm^{-1}; ^{1}H-NMR (DMSO-d_6) δ: 11.30 (s, 1H, NH), 10.00 (d, 1H, NH-CH), 8.20 (d, 1H, H5), 7.15, 7.40 (m, 4H, H8-7-2-2'), 4.70 (d, 1H, H3'), 4.10 (q, 2H, CH_2), 2.65, 2.50 (s, 6H, CH_3 x 2), 1.20 (t, 3H, CH_3). Anal $C_{19}H_{19}BrN_2O_2$ (C, H, N).

9-Chloro-5,11-dimethyl-4-hydroxy-6H-pyrido[3,2-b]carbazole ***8a****. General procedure*

A solution of **8** (5 g, 0.0204 mol), in 40 mL of diphenylether was heated at 200 °C for 10 min. The solid product was filtered and crystallized from acetonitrile to give **8a**. Yield (4.2 g,

69%), as yellow crystals: mp > 280 °C. IR (KBr): ν 3250 (NH), 3420 (OH) cm^{-1}; ^{1}H-NMR (DMSO-d_6) δ: 11.10 (s, 1H, NH), 10.45 (s, 1H, OH), 7.35 (m, 2H, H2-3), 8.00 (d, 1H, H10), 7.40 (dd, 2H, H8-7), 2.65, 2.45 (s, 6H, CH_3 x 2). Anal $C_{17}H_{13}ClN_2O$ (C, H, Cl, N).

9-Bromo-5,11-dimethyl-4-hydroxy-6H-pyrido[3,2-b]carbazole ***9a****. General procedure*

This compound was prepared with the general procedure described for **8a**. Yield (3.8 g, 64%), mp > 280 °C (acetonitrile) as yellow crystals. IR (KBr): ν 3240 (NH), 3440 (OH) cm^{-1}; ^{1}H-NMR (DMSO-d_6) δ: 10.85 (s, 1H, NH), 10.40 (s, 1H, OH), 7.60 (m, 2H, H2-3), 8.30 (d, 1H, H10), 7.40 (dd, 2H, H8-7), 3.02, 2.85 (s, 6H, CH_3 x 2). Anal $C_{17}H_{13}BrN_2O$ (C, H, Br, N).

3-(Chloro-4-hydroxy-2,5,11-trimethyl-6H-pyrido[3,2-b]carbazolyl)propionhydrazine ***10****. General procedure*

A solution of **6i** (1 g, 0.00244 mol) and 15 mL hydrazine hydrate in ethanol (120 mL) was refluxed under nitrogen atmosphere for 2 h. The resulting precipitates were collected and recrystallized from acetonitrile to afford (0.45 g, 47%) of **10**, mp 265 °C. IR (KBr): ν 3240 (NH), 3430 (NH, NH_2, OH), 1670 (CO) cm^{-1}; ^{1}H-NMR (DMSO-d_6) δ: 11.05, 3.75, 9.60 (s, 4H, NH-NH_2-NH), 8.87 (s, 1H, OH), 8.08 (d, 1H, H10), 7.35 (dd, 2H, H8-7), 3.00, 2.80, 2.40 (s, 9H, CH_3 x 3), 2.60, 2.20 (m, 4H, CH_2 x 2). Anal $C_{21}H_{21}ClN_4O_2$ (C, H, Cl, N).

3-(9-Bromo-4-hydroxy-2,5,11-trimethyl-6H-pyrido[3,2-b]-carbazolyl)propionhydrazine ***11***

This compound was prepared with the general procedure described for **10**, yield (0.45 g, 46%), mp > 280 °C (acetonitrile) as yellow crystals. IR (KBr): ν 3240 (NH), 3420 (NH, NH_2, OH), 1660 (CO) cm^{-1}; ^{1}H-NMR (DMSO-d_6) δ: 11.10, 3.65, 9.65 (s, 4H, NH-NH_2-NH), 8.95 (s, 1H, OH), 8.30 (d, 1H, H10), 7.50 (dd, 2H, H8-7), 3.10, 2.90, 2.50 (s, 9H, CH_3 x 3), 2.60, 2.25 (m, 4H, CH_2 x 2). Anal $C_{21}H_{21}BrN_4O_2$ (C, H, N, Br).

3-(9-Chloro-4-hydroxy-5,11-dimethyl-6H-pyrido[3,2-b]carbazolyl)butyrylhydrazine ***12***

This compound was prepared with the general procedure described for **10**, yield (0.45 g, 48%), mp > 280 °C (acetonitrile) as yellow crystals. IR (KBr): ν 3300, 3400, (NH, NH_2, OH), 1645 (CO) cm^{-1}; ^{1}H-NMR (DMSO-d_6) δ: 11.17, 4.10, 9.98 (s, 4H, NH-NH_2-NH), 9.00 (s, 1H, OH), 8.17 (d, 1H, H10), 7.40 (dd, 2H, H8-7), 5.80 (s, 1H, H3), 3.08, 2.97 (s, 6H, CH_3 x 2), 2.63, 2.48, 2.04 (m, 6H, CH_3 x 3). Anal $C_{21}H_{21}ClN_4O_2$ (C, H, N, Cl).

L1210 cytotoxicity determination

Cells cultures and in vitro cytotoxicity determinations were carried out following the procedures described previously [9]. Briefly, a two-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 three following final concentrations : 0.1, 1 and 10 μg/mL. Two drug exposure protocols were used. In the brief exposure, the cells were incubated with drugs for 1 h, washed twice and then cloned in soft agar in multi-well plates. In continuous exposure, drugs were directly added in soft agar. In both cases, cells were cloned at a final concentrations of 40 000 cells/mL (12 000 cells/well). Colonies were counted after 5–7 days 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 triplicated-treated cultures was expressed as a percentage of the average colony number in the untreated controls. A compound was considered active if it reduced colony formation to 50% or less of the control value. The reduction of colony formation by L1210 cells is noted as follows: – inactive at all doses (colony number above 50% of control); ± weakly active at 10 μg/mL (colony number between 50 and 30% of control); + active at 10 μg/mL (colony number below 30% of control); ++ fully active at 10 μg/mL (total inhibition of colony formation); and +++ active at 1 μg/mL (colony number below 50% of control). *N*2-Methyl-9-hydroxyellipticinium acetate (NMHE) was used as a reference compound.

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References

1 Juret P, Heron JF, Couette JE, Delozier I, Le Talaer JY (1982) *Cancer Treat Rep* 66, 1916–1919
2 Mucci-Lorusso P, Polin L, Biernot LA, Valeriote FA, Corbett TH (1990) *Invest New Drugs* 8, 253–261
3 Balosso J, Giocanti N, Favaudon V (1991) *Cancer Res* 51, 3204–3211
4 Moinet-Hedin V, Tabka T, Gauduchon P et al (1993) *Eur J Med Chem* 28, 721–726
5 Cranwell PA, Saxton JE (1962) *J Chem Soc* 3482–3495
6 Dalton LK, Demerac S, Elmes BC, Loder JW, Swan JM, Teitei T (1967) *Austr J Chem* 20, 2715–2720
7 Dalton LK, Teitei T (1968) *Austr J Chem* 21, 2053–2060
8 Lancelot JC, Rault S, Robba M et al (1986) XXII[es] Rencontres internationales de chimie thérapeutique, Clermont-Ferrand
9 Tabka T, Letois B, Lancelot JC et al (1989) *Eur J Med Chem* 24, 605–610
10 Viossat B, Nguyen-huy D, Lancelot JC, Benazeth S, Rault S, Robba M (1992) *Chem Pharm Bull* 35, 1724–1733
11 Nguyen-huy D, Viossat B, Lancelot JC, Robba M (1984) *Acta Crystallogr Sect C* 40, 1602–1608
12 Robba M, Lancelot JC, Rault S, Gauduchon P, Le Talaer JY (1990) French Patent, RCI, 617/MR/CD, 90 09 688
13 Paoletti C, Cros S, Dat-Xuong N, Lecointe P, Moisand A (1979) *Chem Biol Interact* 25, 45–58
14 Acton EM, Narayanan VL, Risbood PA, Shoemaker RH, Vistica DT, Boyd MR (1994) *J Med Chem* 37, 2185–2189
15 Shoemaker RH, Balaschak RS, Alexander RS, Boyd MR (1994) *Proc Am Assoc Cancer Res* 35, 399
16 Lancelot JC, Rault S, Robba M, Nguyen-huy D (1987) *Chem Pharm Bull* 35, 425–433