Synthesis and Biological Evaluation of 6-(9-Hydroxy-5-methyl (and 5,6-Dimethyl)-6*H*-pyrido[4,3-*b*]carbazol-1-yl)picolinic Amides as New Olivacine Derivatives

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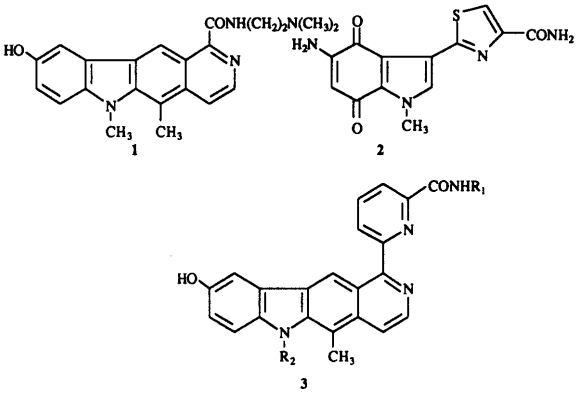
Starting from 2-(6-methoxy-1-methylcarbazol-2-yl)ethylamine and diethyl-2,6-pyridine dicarboxylate, the title compounds were obtained through five or six steps. The new compounds retained significant cytotoxicity towards various tumor cell lines, but *in vivo* studies on murine P388 leukemia, B16 melanoma and Lewis lung carcinoma showed a lowered antitumor activity with respect to that of the related olivacine lead compound 1.

Key words carboxamido olivacine derivative; 1-substituted-6*H*-pyrido[4,3-*b*]carbazole; cytotoxicity; antitumor evaluation

In recent papers,[2-4)] we described the synthesis and antitumor activity of the new carboxamido olivacine derivative **1**. This compound, which is a topoisomerase II inhibitor,[5)] was selected for further pharmacological and toxicological studies and will enter phase 1 clinical trial in the near future.

Compound **2** which is an indolequinone with a carboxamido-thiazole group at its 3-position, was also reported as a potent topoisomerase II inhibitor.[6,7)] As a part of our search for new, and more specific antitumor drugs, we focused on the as-yet unknown olivacine picolinic amide **3**, which is somewhat related to both **1** and **2**. In this paper, we report on the synthesis and biological evaluation of the series **3**.



Synthetic Chemistry The starting compound 2-(6-methoxy-1-methyl-9*H*-carbazol-2-yl)ethylamine (**4**) has already been described.[2)] It was allowed to react with diethyl-2,6-pyridine dicarboxylate **5** and cyclization of the resulting amide **6**, with phosphorous oxychloride in boiling toluene, led to ethyl 6-(3,4-dihydro-9-methoxy-5-methyl-6*H*-pyrido[4,3-*b*]carbazol-1-yl)picolinate (**7**), after hydrolysis, basification with concentrated aqueous ammonia and usual treatment. Dehydrogenation of **7** to **8** took place in boiling mesitylene in the presence of 10% palladized charcoal (73% yield), and *N*-methylation of this last compound was performed by using an excess of dimethylcarbonate in dimethylformamide, in the presence of potassium carbonate and 18-crown-6. This reaction was accompanied by a partial transesterification which mainly provided the methyl ester **8b** (24%), besides the ethyl ester **8c**. 9-*O*-Demethylation of the esters **8a** and **8b** was performed with boron tribromide, to give **9a** and **9b**, respectively. Finally, by heating these esters in boiling 2-dimethylaminoethylamine or 3-dimethylaminopropylamine in excess, the carboxamides **3a—c** were obtained in high yields (Chart 1).

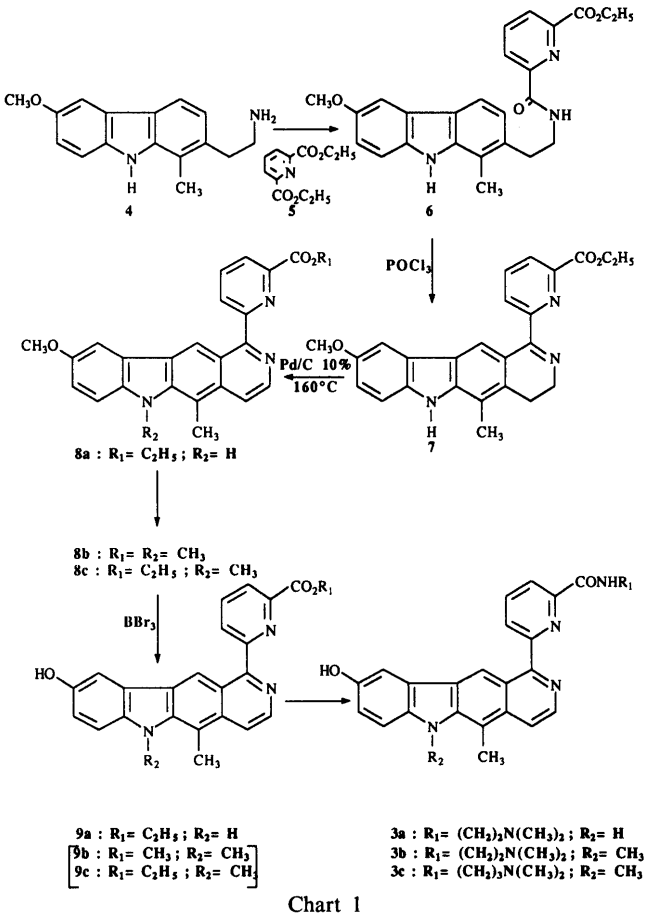


Chart 1

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Experimental

Melting points (mp, uncorrected) were determined with an electrothermal IA 9200 apparatus. All ^{1}H-NMR spectra, were recorded on a Bruker AC 200 apparatus. Elemental analyses were performed by Service Central de Microanalyses du CNRS, 91190 Gif-sur-Yvette, France.

Ethyl 6-[[*N*-[2-(6-Methoxy-1-methylcarbazol-2-yl)ethyl]carboxamido]]-2-picolinate (6) A mixture of 2-(6-methoxy-1-methylcarbazol-2-yl)ethylamine **4** (1.5 g, 6 mmol) and diethyl-2,6-pyridine dicarboxylate **5** (3.8 g, 17 mmol) in xylene (30 ml) was heated at reflux under stirring for 10 h. The mixture was evaporated to dryness under reduced pressure, and the solid residue was chromatographed on a silica gel column. Elution with pure methylene chloride provided the recovered aminoethyl carbazole **4**, and methylene chloride–ethyl alcohol mixture 95 : 5 afforded the expected amide **6**. It was taken up in hexane and filtered to give beige crystals (1.56 g, 61%) mp 87 °C. *Anal.* Calcd for $C_{25}H_{25}N_3O_4$: C, 69.59; H, 5.84; N, 9.74. Found: C, 69.18; H, 6.15; N, 9.35. ^{1}H-NMR (DMSO-d_6) δ: 1.37 (t, 3H, $-CH_2C\underline{H}_3$), 2.59 (s, 3H, 1-CH_3), 3.09 (m, 2H, β-CH_2), 3.60 (m, 2H, α-CH_2), 3.86 (s, 3H, 6-OCH_3), 4.42 (q, 2H, $-C\underline{H}_2CH_3$), 7.00 (m, 2H, 3-H + 7-H), 7.41 (d, 1H, 8-H, J_{7-8} = 8.0 Hz), 7.63 (d, 1H, 5-H, J_{5-7} = 2.5 Hz), 7.87 (d, 1H, 4-H, J_{3-4} = 7.8 Hz), 8.27 (m, 3H, pyr.), 8.73 (t, 1H, β-NH), 10.88 (s, 1H, 9-NH).

Ethyl 6-(3,4-Dihydro-9-methoxy-5-methyl-6*H*-pyrido[4,3-*b*]carbazol-1-yl)picolinate (7) The preceding amide **6** (1.5 g, 3.48 mmol) was dissolved in boiling toluene (60 ml) and treated dropwise with phosphorous oxychloride (5 ml). The mixture was refluxed for 10 h and evaporation under reduced pressure afforded a residue, which was taken up in water (50 ml), basified with concentrated aqueous ammonia and extracted with methylene chloride. Evaporation of the solvent provided a solid residue, which was recrystallized from toluene to give pale yellow crystals (0.8 g, 56%), mp 248 °C. *Anal.* Calcd for $C_{25}H_{23}N_3O_3$: C, 72.62; H, 5.61; N, 10.16. Found: C, 72.41; H, 5.68; N, 10.01. ^{1}H-NMR (DMSO-d_6) δ: 1.33 (t, 3H, $-CH_2C\underline{H}_3$), 2.58 (s, 3H, 5-CH_3), 3.12 (t, 2H, 4-CH_2), 3.80 (s, 3H, 9-OCH_3), 3.90 (t, 2H, 3-CH_2), 4.40 (q, 2H, $-C\underline{H}_2CH_3$), 7.07 (dd, 1H, 8-H, J_{8-10} = 8.8 Hz, J_{7-8} = 2.3 Hz), 7.49 (d, 1H, 7-H, J_{7-8} = 8.7 Hz), 7.65 (d, 1H, 10-H, J_{8-10} = 2.3 Hz), 8.14 (s, 1H, 11-H), 8.33 (m, 3H, pyr.), 11.74 (s, 1H, 6-NH).

Ethyl 6-(9-Methoxy-5-methyl-6*H*-pyrido[4,3-*b*]carbazol-1-yl)picolinate (8a) The ethyl ester **7** (5 g, 12 mmol) was refluxed in mesitylene (1 l) in the presence of 10% palladized charcoal (0.75 g) for 20 h. The catalyst was filtered off and washed with boiling ethyl alcohol, and the filtrate and washing were evaporated to dryness under reduced pressure. The solid residue was chromatographed on a silica gel column with methylene chloride–ethyl alcohol mixture (97 : 3). The eluate was evaporated and the solid residue was taken up in ethyl acetate to give yellow crystal (3.6 g, 73%), mp 223 °C. *Anal.* Calcd for $C_{25}H_{21}N_3O_3$: C, 72.98; H, 5.14; N, 10.21. Found: C, 72.66; H, 5.35; N, 10.02. ^{1}H-NMR (DMSO-d_6) δ: 1.38 (t, 3H, $-CH_2C\underline{H}_3$), 2.92 (s, 3H, 5-CH_3), 3.88 (s, 3H, 9-OCH_3), 4.48 (q, 2H, $-C\underline{H}_2CH_3$), 7.20 (dd, 1H, 8-H, J_{8-10} = 8.7 Hz, J_{7-8} = 2.5 Hz), 7.50 (d, 1H, 7-H, J_{7-8} = 8.7 Hz), 7.76 (d, 1H, 10-H, J_{8-10} = 2.4 Hz), 8.11 (d, 1H, 4-H, J_{3-4} = 6.0 Hz), 8.31 (m, 3H, pyr.), 8.56 (d, 1H, 3-H, J_{3-4} = 6.0 Hz), 9.47 (s, 1H, 11-H), 11.29 (s, 1H, 6-NH).

Methyl (and Ethyl) 6-(9-Methoxy-5,6-dimethyl-6*H*-pyrido[4,3-*b*]carbazol-1-yl)picolinates (8b and 8c) A mixture of ethyl ester **8a** (0.2 g, 0.49 mmol), finely powdered dry potassium carbonate (0.16 g), dimethylcarbonate (9 ml), dimethylformamide (0.6 ml) and 18-crown-6 (0.05 g) was heated at reflux under stirring for 20 h. After evaporation to dryness, the residue was taken up in water and the solid was collected and air-dried. It was chromatographed on a silica gel column with methylene chloride–ethyl alcohol (95 : 5, v/v) mixture to give two products:

a) The more mobile one, which corresponded to the ethyl ester **8c** (30 mg, 14%), was obtained as yellow crystals, mp 218 °C. *Anal.* Calcd for $C_{26}H_{23}N_3O_3$: C, 73.39; H, 5.45; N, 9.88. Found: C, 73.13; H, 5.38; N, 9.96. ^{1}H-NMR (DMSO-d_6) δ: 1.47 (t, 3H, $-CH_2C\underline{H}_3$), 2.96 (s, 3H, 5-CH_3), 3.66 (s, 3H, 9-OCH_3), 3.99 (s, 3H, 6-CH_3), 4.22 (q, 2H, $-C\underline{H}_2CH_3$), 7.00 (dd, 1H, 8-H, J_{8-10} = 2.5 Hz, J_{7-8} = 8.9 Hz), 7.38 (d, 1H, 7-H, J_{7-8} = 8.9 Hz), 7.55 (d, 1H, 10-H, J_{8-10} = 2.5 Hz), 8.00 (m, 3H, pyr.), 8.14 (d, 1H, 4-H, J_{3-4} = 6.0 Hz), 8.35 (d, 1H, 3-H, J_{3-4} = 6.2 Hz), 9.38 (s, 1H, 11-H).

b) The less mobile methyl ester **8b** resulting from partial transesterification (50 mg, 24%) was obtained as yellow crystals, mp 252 °C. *Anal.* Calcd for $C_{25}H_{21}N_3O_3$: C, 72.98; H, 5.14; N, 10.21. Found: C, 72.62; H, 5.46; N, 9.82. ^{1}H-NMR (DMSO-d_6) δ: 2.95 (s, 3H, 5-CH_3), 3.67 (s, 3H, 9-OCH_3), 3.77 (s, 3H, $-CO_2CH_3$), 3.99 (s, 3H, 6-CH_3), 7.02 (dd, 1H, 8-H, J_{8-10} = 2.5 Hz, J_{7-8} = 8.7 Hz), 7.37 (d, 1H, 7-H, J_{7-8} = 8.9 Hz), 7.54 (d, 1H, 10-H, J_{8-10} = 2.5 Hz), 8.03 (m, 3H, pyr.), 8.18 (d, 1H, 4-H, J_{3-4} = 6.0 Hz), 8.36 (d, 1H, 3-H, J_{3-4} = 6.1 Hz), 9.43 (s, 1H, 11-H). For the preparation of the amino derivatives **3b** and **3c**, the crude mixture of **8b** + **8c** was used without further purification.

Demethylation of the Ester 8a to the 9-Hydroxy Ester (9a) The 9-methoxy ester **8a** (1.5 g, 3.76 mmol) was dissolved in dry methylene chloride (176 ml) maintained under an argon atmosphere, and the mixture was cooled to −78 °C. A solution of 1 M boron tribromide in methylene chloride (37 mmol) was added dropwise and stirring at −78 °C was continued for 4 h. The mixture was allowed to reach room temperature for 1 h, poured in water (50 ml), basified with concentrated aqueous ammonia and stirred at room temperature for 1 h. Since decomplexation was incomplete, the mixture was heated at reflux with ethyl alcohol (15 ml) for a further 1 h and the precipitate was collected, washed with water and air-dried. The aqueous layer was extracted with methylene chloride, dried over magnesium sulfate and evaporated to dryness. The residue and the collected solid were combined and purified by column chromatography over a silica gel column. Elution with methylene chloride–ethyl alcohol 98 : 2 v/v gave the pure hydroxy ester, after evaporation of the appropriate fractions, as yellow crystals (23% yield), mp > 260 °C. *Anal.* Calcd for $C_{24}H_{19}N_3O_3 \cdot H_2O$: C, 69.38; H, 5.10; N, 10.12. Found: C, 69.08; H, 4.91; N, 10.03. ^{1}H-NMR (DMSO-d_6) δ: 1.41 (t, 3H, $-CH_2C\underline{H}_3$), 2.90 (s, 3H, 5-CH_3), 4.48 (q, 2H, $-C\underline{H}_2CH_3$), 7.06 (dd, 1H, 8-H, J_{8-10} = 2.5 Hz, J_{7-8} = 8.7 Hz), 7.40 (d, 1H, 7-H, J_{7-8} = 8.5 Hz), 7.53 (d, 1H, 10-H, J_{8-10} = 2.5 Hz), 8.10 (d, 1H, 4-H, J_{3-4} = 5.9 Hz), 8.31 (m, 3H, pyr.), 8.37 (d, 1H, 3-H, J_{3-4} = 5.9 Hz), 9.13 (s, 1H, 9-OH), 9.43 (s, 1H, 11-H), 11.18 (s, 1H, 6-NH). The hydroxy ester **9a** was obtained in low yield. In view of the better overall yields obtained in the preparation of the amides **3a**—**c** from **8a** and **8b** + **8c**, this can be explained in terms of difficulty in taking the decomposition–decomplexation to completion.

Synthesis of the Amides 3a—c Starting from the ester **8a** or the mixture **8b** + **8c** (10 mmol), the crude solid obtained by aqueous decomposition after boron tribromide demethylation (see above) was collected, air-dried and dissolved in the appropriate amine (10 ml). The mixture was heated at reflux for 10 h, and excess amine was evaporated. The residue was taken up in water, and extracted with methylene chloride. The organic layer was dried over magnesium sulfate and evaporation of the solvent provided a residue, which was chromatographed over an alumina column. Evaporation of the appropriate methylene chloride–ethyl alcohol mixture 95 : 5 v/v eluates gave a solid residue, which was taken up in diethyl ether and filtered to provide yellow crystals.

3a: Yield 25.5%, mp > 260 °C. *Anal.* Calcd for $C_{26}H_{25}N_5O_2 \cdot 0.5\,H_2O$: C, 69.62; H, 5.84; N, 15.61. Found: C, 69.83; H, 5.91; N, 15.34. ^{1}H-NMR (DMSO-d_6) δ: 1.98 (s, 6H, $-N(CH_3)_2$), 2.40 (t, 2H, β-CH_2), 2.91 (s, 3H, 5-CH_3), 3.42 (q, 2H, α-CH_2), 7.05 (dd, 1H, 8-H, J_{8-10} = 2.5 Hz, J_{7-8} = 8.8 Hz), 7.40 (d, 1H, 7-H, J_{7-8} = 8.5 Hz), 7.51 (d, 1H, 10-H, J_{8-10} = 2.5 Hz), 8.12 (d, 1H, 4-H, J_{3-4} = 5.9 Hz), 8.25 (m, 3H, pyr.), 8.55 (m, 2H, 3H + NH), 8.95 (s, 1H, 11-H), 9.08 (s, 1H, 9-OH), 11.15 (s, 1H, 6-NH).

3b: Yield 37%, mp > 260 °C. *Anal.* Calcd for $C_{27}H_{27}N_5O_2 \cdot 0.5H_2O$: C, 69.89; H, 5.93; N, 15.38. Found: C, 70.12; H, 6.11; N, 15.31. ^{1}H-NMR (DMSO-d_6) δ: 1.96 (s, 6H, $-N(CH_3)_2$), 2.39 (t, 2H, β-CH_2), 3.16 (s, 3H, 5-CH_3), 3.44 (q, 2H, α-CH_2), 4.18 (s, 3H, 6-NCH_3), 7.07 (dd, 1H, 8-H, J_{8-10} = 2.5 Hz, J_{7-8} = 8.8 Hz), 7.48 (m, 2H, 7-H + 10-H), 8.22 (m, 4H, 4-H + pyr.), 8.56 (m, 2H, 3-H + NH), 9.00 (s, 1H, 11-H), 9.18 (s, 1H, 9-OH).

3c: Yield 26%, mp > 260 °C. *Anal.* Calcd for $C_{28}H_{29}N_5O_2$: C, 71.92; H, 6.25; N, 14.98. Found: C, 71.72; H, 6.32; N, 15.06. ^{1}H-NMR (DMSO-d_6) δ: 1.69 (m, 2H, β-CH_2), 1.99 (s, 6H, $-N(CH_3)_2$), 2.34 (t, 2H, γ-CH_2), 3.17 (s, 3H, 5-CH_3), 3.41 (m, 2H, α-CH_2), 4.18 (s, 3H, 6-NCH_3), 7.05 (dd, 1H, 8-H, J_{8-10} = 2.5 Hz, J_{7-8} = 8.7 Hz), 7.50 (m, 2H, 7-H + 10-H), 8.19 (d, 1H, 4-H, J_{3-4} = 5.9 Hz), 8.26 (m, 3H, pyr.), 8.58 (d, 1H, 3-H, J_{3-4} = 5.9 Hz), 8.85 (m, 1H, –NH), 9.19 (s, 1H, 11-H), 9.95 (s, 1H, 9-OH).

Biological Studies Cell Culture and Cytotoxicity: Three murine (L1210 leukemia, B16 melanoma, Lewis lung carcinoma) and three human (MCF7 breast adenocarcinoma, A-549 lung carcinoma, KB-3-1 epidermoid carcinoma) cell lines were used. Cells were cultivated in RPMI 1640 medium (Gibco) supplemented with 10% fetal calf serum, 2 mM L-glutamine, 100 units/ml penicillin, 100 μg/ml streptomycin and 10 mM HEPES buffer (pH 7.4). Cytotoxicity was measured by the microculture tetrazolium assay as described.[8] Cells were exposed to graded con-

Table 1. Cytotoxicity of Compounds **3a—c** on Various Tumor Cell Lines, Compared to Compound **1** and Adriamycin (ADR)

	IC_{50} (nM ± S.E.M.)					
	L1210	B16	LLC	A549	KB-3-1	MCF7
3a	661.9 ± 151.8	77.8 ± 7.4	76.3 ± 2.9	947.6 ± 133.5	189.3 ± 133.7	2800 ± 156
3b	50.1 ± 11.2	5.9 ± 1.9	22.3 ± 9.6	95.4 ± 40.1	29.6 ± 3.1	238.8 ± 20.3
3c	55.7 ± 16.7	5.9 ± 2.6	20.1 ± 8.4	101.4 ± 18.2	26.3 ± 5.1	144.6 ± 12.0
1	8.4 ± 0.7	5.1 ± 0.5	23.5 ± 5.5	30.5 ± 4.5	29.9 ± 2.5	75.8 ± 11.2
ADR	32.6 ± 1.8	6.8 ± 0.7	19.3 ± 3.0	42.2 ± 4.4	16.5 ± 1.4	41.5 ± 6.5

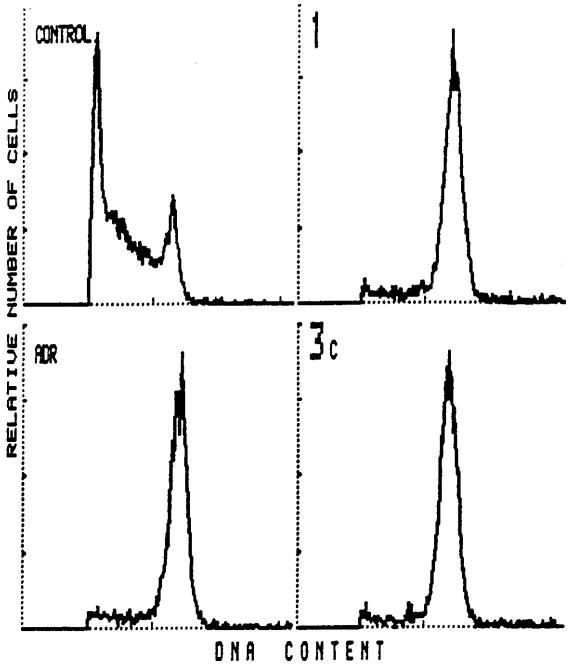

Fig. 1. Modification of the Typical DNA Histogram of L1210 Cells Treated with 50 nM **1**, 100 nM ADR, or 250 nM **3c** for 21 h

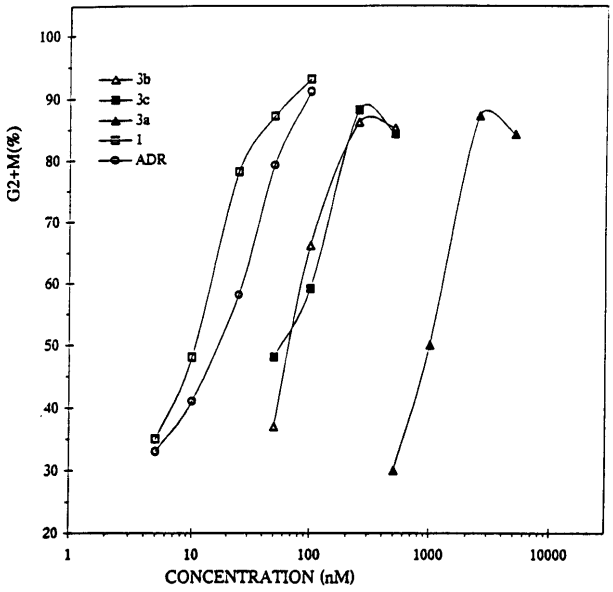

Fig. 2. Effect of Compounds **1**, **3a—c** and ADR on the Accumulation of L1210 Cells in the G2 + M Phase of the Cell Cycle

Table 2. Antitumor Activity against P388 Leukemia

Compound	Optimal dose[a] (mg/kg)	Schedule	Median % T/C survival (LTS)[b]
3a	20	J1	130
	20	J1, 5, 9	110
3b	40	J1	151
	40	J1, 5, 9	207
3c	80	J1	189
	40	J1, 5, 9	213
1	120	J1	301
	80	J1, 5, 9	>631 (5/6)

The compounds were administered i.v. to P388-bearing mice on J1 or J1, 5, 9 as indicated. *a*) Dose giving the best antitumor activity without toxicity. *b*) Long-term survivors, scored on day 60.

centrations of a test drug (nine serial dilutions in triplicate) for four doubling times (48 h for L1210, 168 h for MCF7 and 96 h for the other lines). Results were expressed as IC_{50}, the concentration which reduced by 50% the optical density of treated cells with respect to untreated controls, ± standard error of the mean (S.E.M.).

For the cell cycle analysis, L1210 cells (5×10^5 cells/ml) were incubated for 21 h with various concentrations of drugs. Cells were then fixed in 70% ethanol (v/v), washed and incubated in phosphate buffered saline (PBS) containing 100 μg/ml RNase and 25 μg/mg propidium iodide for 30 min at 20 °C. For each sample, 10000 cells were analyzed on an ATC3000 flow cytometer (Brucker, Wissembourg, France).

Antitumor Activity: The antitumor activity of the compounds was evaluated in three experimental models, P388 leukemia, Lewis lung carcinoma and B16 melanoma, all provided by the NCI, Frederick, U.S.A. P388 cells were inoculated i.p. (10^6 cells/mouse) into B6D2F1 mice (Iffa Credo) on day 0. The dichlorhydrate salts of **1** and **3** were dissolved and diluted in water and injected i.v. on day 1 or days 1, 5 and 9. The results are expressed in terms of T/C % for survival (median survival time of treated animals/median survival time of control animals, × 100). For the i.p. B16 melanoma, 0.5 ml of a tumor suspension (1 g of tumor in 10 ml of 0.9% NaCl) was injected i.p. on day 0, and compounds were administered i.v. on days 2, 6 and 10. Results are expressed as T/C % for survival. For the s.c. Lewis lung carcinoma, a tumor fragment of approximately 30 mg was grafted s.c. into B6D2F1 mice on day 0 and drugs were administered i.v. on days 3, 7 and 11. The tumor volumes were measured on day 20, and the results are expressed as T/C % for tumor volume (median tumor volume in treated animals/median tumor volume in control animals × 100) and also as T/C % for survival.

Results

Cytotoxicity Compounds **3a—c** were evaluated for cytotoxicity towards various tumor cell lines, in comparison with olivacine derivative **1** as the reference and lead compound. As shown in Table 1, *in vitro* cytotoxicity of the new series was lower than that of compound **1**. Compound **3a** was the least potent derivative, being at least 10-fold less potent than **1**. Compound **3c** retained a significant cytotoxicity, of the same order as that of **1**, on B16, LLC, A549, KB-3-1 and MCF7 cell lines (Table 1). The three derivatives were found to cause L1210 cells to accumulate in the G2 + M phase of the cell cycle (Fig. 1), as is the case for **1** and ADR. Moreover, the good

Table 3. Antitumor Activity against i.p. B16 Melanoma

Compound	Optimal dose[a] (mg/kg)	Median % T/C survival
3a	20	102
3b	40	111
3c	40	116
1	40	140

The compounds were administered i.v. to B16-bearing mice on days 2, 6 and 10. *a*) Dose giving the best antitumor activity without toxicity.

Table 4. Antitumor Activity against s.c. Lewis Lung Carcinoma

Compound	Optimal dose[a] (mg/kg)	Median % T/C	
		Tumor volume	Survival (LTS)[b]
3a	20	112	96
3b	40	69	130
3c	40	27	139
1	40	0	>288 (7/7)

The compounds were administered i.v. to LLC-bearing mice on days 3, 7 and 11. *a*) Dose giving the best antitumor activity without toxicity. *b*) Long-term survivors on day 90.

correlation between cytotoxicity and potency in accumulating L1210 cells in the G2+M phase, previously found in the series of **1**[2] was also observed with **1** and **3a—c**, as shown in Fig. 2: **3c** is the most potent of the new compounds, but is less active than **1**. As discussed in our previous paper, this relationship suggests that the three compounds and **1** share the same mechanism of action.

Antitumor Activity Compounds **3b** and **3c** were significantly active against P388 leukemia, but markedly less so than **1**, which gave five long-term survivors among six mice when administered at 80 mg/kg on days 1, 5 and 9. The three compounds, administered i.v. were inactive against B16 melanoma, unlike **1**, which was moderately active, increasing by 40% the survival of treated mice. Against s.c. Lewis lung carcinoma, **3b** and **3c** were moderately active, but dramatically less so than **1**, which was curative for 100% of the animals when administered i.v. on days 3, 7 and 11. Again, **3c** was the most efficient of the three compounds, reducing by 73% the tumor growth and increasing by 39% the life span of treated animals (Table 4).

Discussion and Conclusion

6-(9-Hydroxy-5-methyl-6*H*-pyrido[4,3-*b*]carbazol-1-yl)picolinic amides (**3**), which are somewhat related to the topoisomerase II inhibitors **1** and **2**, were designed as new olivacine analogues and synthesized *via* five or six step sequence. The *in vitro* and *in vivo* test results may be summarized as follows.

i) The cytotoxicity of the new compounds towards various tumor cell lines is generally lower than that of **1**.

ii) Significant *in vivo* activity was maintained in the P388 model with the *N*-methylated compounds **3b** and **3c**, but they were inactive against B16 melanoma and only moderately active against Lewis lung carcinoma.

iii) As in the olivacine series **1**, *N*-methylation of the pyrrole nitrogen atom resulted in higher *in vitro* and *in vivo* activities than for the corresponding NH compound.

Thus, these modifications did not lead to promising results.

References

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