Acta Poloniae Pharmaceutica – Drug Research, Vol. 62 No. 3 pp. 207–212, 2005 ISSN 0001-6837
Polish Pharmaceutical Society

DRUG SYNTHESIS

AN ALTERNATIVE WAY OF THE SYNTHESIS OF 1-SUBSTITUTED 9-METHOXY-5-METHYL-6*H*-PYRIDO[4,3-*b*]CARBAZOLE DERIVATIVES

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Abstract: A keystone of this work was a modification of synthesis of the title compounds, which were used as substrates for the preparation of amides **5**, 9-methoxyolivacine (**4a**) and ethyl 9-methoxy-5-methyl-6*H*-pyrido[4,3-*b*]carbazole-1-carboxylate (**4b**) were obtained in good overall yields (**4a** – 72%, and **4b** – 31%) on alternative ways of the synthesis. The pilot results of the cytostatic activity of iminium salts **12a** (IC_{50} = 8 μM) and **12b** (IC_{50} = 2 μM) were determined on L1210 mouse leukaemia cells.

Keywords: olivacine; 1-substituted-6*H*-pyrido[4,3-b]carbazole; cytotoxicity

Since 1958, when alkaloids Ellipticine **1** and its isomer Olivacine **2** were isolated, and theirs structures and antineoplastic activity were confirmed, many different methods of synthesis of pyrido [4,3-*b*] carbazole derivatives have been devised.

Many structural analogues of both the alkaloids have been synthesized, and their antitumor properties have been studied. So far, 9-hydroxy-2-methylelliptinium acetate **3** has been used in clinics for the treatment of advanced breast cancer (1, 2), and many other synthetic analogues of the two alkaloids have been studied in preclinical and clinical trials (3, 4).

In the earlier works, the syntheses of 9-methoxyolivacine **4a**, and esters **4b** and **4c** were reported (5, 6). The last ones were the substrates for the preparation of amide **5**.

These amides showed a very strong antiproliferative activity against many cancer lines. The biological activity of these compounds was comparable, and in some cases even higher, than the reference Adriamycin. One of these analogues is amide **5a**, that entered into phase II of a clinical trial (7). Also 1,2-dihydroellipticines **6**, prepared by Jurayj at al. (8), showed cytotoxicities for the different cell lines.

All these facts prompted us to undertake a study on an alternative way for the preparation of the title compounds according to Scheme 1 given below, and at the same time, we wanted to examine a cytotoxic activity of the new 1-substituted 2-benzyl-3,4-dihydro-9-methoxypyrido [4,3-*b*] carbazole derivatives. A keystone of the above modification was elimination of the debenzylation reaction of compound **7** to give **8**, and the attainment on this way of synthesis a possibly better total yield of 9-methoxyolivacine **4a** as well as ester **4b**.

EXPERIMENTAL

Material and methods

Melting points were determined on a Köfler apparatus and were uncorrected; ^{1}H NMR spectra were recorded on a Bruker 300 spectrometer at 300.14 MHz, using TMS as an internal standard.

Column chromatography was carried out on silica gel (Merck Kieselgel 100). All of the newly obtained compounds were analyzed for C, H, N, and the analytical results were within ±0.4% of the theoretical values.

General procedures

The starting 2-[2-(benzylamino)ethyl]-6-methoxy-1-methylcarbazole (**7**), 2-(6-methoxy-1-methylcarbazol-2-yl) ethylamine (**8**), 2-[2-(ethoxalyl--amino) ethyl]-6-methoxy-1-methylcarbazole (**9b**), ethyl 3,4-dihydro-9-methoxy-5-methyl-6*H*-pyrido [4,3-*b*] carbazole-1-carboxylate (**10b**) and ethyl 9-methoxy-5-methyl-6*H*-pyrido [4,3-*b*] carbazole-1-carboxylate (**4*b***) (Scheme 1) were obtained according to the procedure devised by Jasztold-Howorko et al. (6).

2-[2-(Acetylamino) ethyl]-6-methoxy-1-methylcar-

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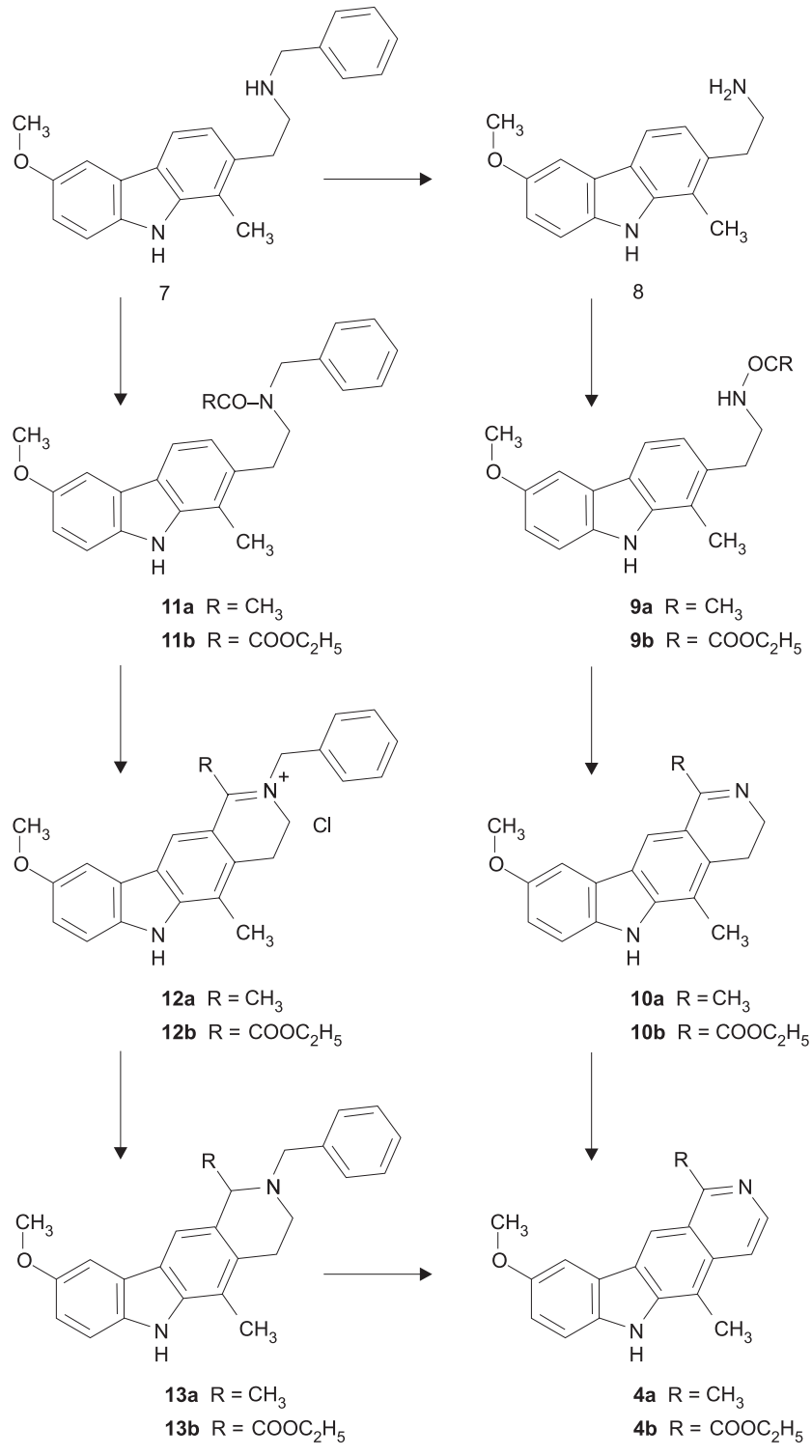
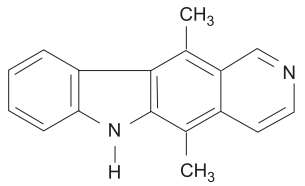

7
8
11a R = CH3
11b R = COOC2H5
9a R = CH3
9b R = COOC2H5
12a R = CH3
12b R = COOC2H5
10a R = CH3
10b R = COOC2H5
13a R = CH3
13b R = COOC2H5
4a R = CH3
4b R = COOC2H5

Scheme 1.



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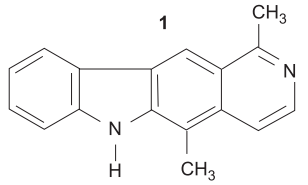

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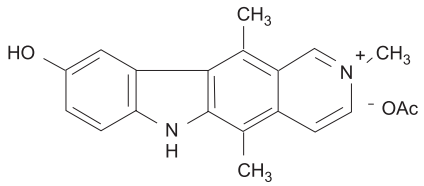
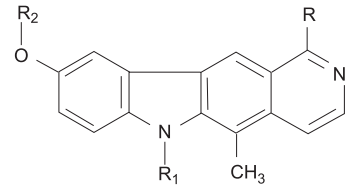
Figure 1.



3

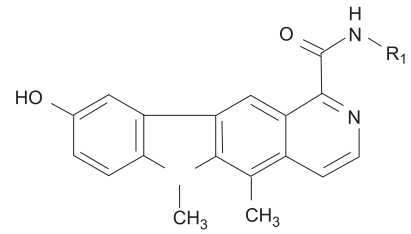
Figure 2.



4 a R = R_2 =CH_3, R_1 = H

4 b R = $COOC_2H_5$, R_1 = H, R_2 = CH_3

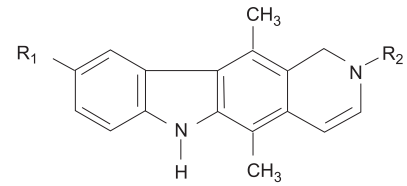
4 b R = $COOC_2H_5$, R_1 =CH_3, R_2 = H



5 a R_1 = $CH_2CH_2N(CH_3)_2$

5 b R_1 = $CH_2CH_2CH_2N(CH_3)_2$

Figure 3.



6

Figure 4.

bazole **(9a)**, 3,4-dihydro-1,5-dimethyl-9-methoxy--6*H*-pyrido [4,3-*b*] carbazole **(10a)** and 9-methoxyolivacine **(4a)** were obtained according to the procedure published by Jasztold-Howorko et al. (5).

2-[2-(N-ACETYL-N-BENZYLAMINO) ETHYL]-6-METHOXY-1-METHYLCARBAZOLE **(11a)**

Secondary amine **7** (0.69 g, 2 mmole) was stirred for 2 h in a mixture made of acetic anhydride and pyridine (1: 1) (10 ml). The evaporation to dryness under reduced pressure left a residue, which was taken up into water (100 ml). The solid was collected by filtration and washed with water, and recrystallized from ethanol to give 0.76 g (98%) of compound **11a**, m. p. = 156°C. Elemental analysis for **11a** $C_{25}H_{26}N_2O_2$: M.w. 386.5; Calc.: C, 77.69%; H, 6.78%; N, 7.25%. Found: C, 77.7%; H, 6.6%; N, 7.1%. ^{1}H NMR ($CDCl_3$) exhibited two rotamers, δ: 2.08 and 2.17 (2s, 3H, -CO**C**$\mathbf{H_3}$), 2.42 and 2.49 (2s, 3H, **1-C**$\mathbf{H_3}$), 2.98 (m, 2H, **α-C**$\mathbf{H_2}$), 3.43 (m, 3H, **β--C**$\mathbf{H_2}$), 3.93 (s, 3H, **6-**OCH_3), 4.36 and 4.59 (2s, 2H, **-C**$\mathbf{H_2}$-C_6H_5), 6.97 (d, J = 8.1 Hz, 1H, **3-H**), 7.10 (dd, J = 7.6 Hz, J = 1.8 Hz, 1H, **7-H**), 7.17-7.37 (m, 5H, CH_2-**C**$\mathbf{_6H_5}$), 7.48 (d, J = 7.8 Hz, 1H, **8-H**), 7.69 (d, J = 1.8 Hz, 1H, **5-H**), 7.93 (d, J = 8.1 Hz, 1H, **4-H**), 8.20 (br s, 1H, **9-H**).

2-[2-(N-BENZYL-N-ETHOXALYLAMINO)]-6-METHOXY-1-METHYLCARBAZOLE (**11b**)

A mixture of benzylamino derivative **7** (0.69 g, 2 mmole), diethyl oxalate (5 mL) and toluene (10

mL) was heated under a reflux condenser with stirring for 5 h, and then it was evaporated to dryness under reduced pressure, with leaving the residue which was taken up into water (100 mL). The solid was collected by filtration, washed with water, and recrystallized from ethanol to give 0.66 g (74.2%) of compound **11b**, m. p. = 143.5°C. Elemental analysis for **11b** $C_{27}H_{28}N_2O_4$: M. w. 444.5; Calc.: C, 72.95%; H, 6.35%; N, 6.30%. Found: C, 73.2%; H, 6.4%; N, 6.3%. ^{1}H NMR ($CDCl_3$) exhibited two rotamers, δ: 1.28 (t, 3H, $COOCH_2\mathbf{CH_3}$), 2.39 and 2.47 (2s, 3H, **1-CH_3**), 3.03 (m, 2H, **α-CH_2**), 3.48 (m, 2H, **β-CH_2**), 3.91 (s, 3H, **6-OCH_3**), 4.24 (m, 2H, $COO\mathbf{CH_2}CH_3$), 4.38 and 4.61 (2s, 2H, **-CH_2-**C_6H_5), 6.86 (d, J = 8.0 Hz, 1H, **3-H**), 7.02 (dd, J = 7.8 Hz, J = 2.0 Hz, 1H, **7-H**), 7.20-7.31 (m, 5H, CH_2-**C_6H_5**), 7.44 (d, J = 8.0 Hz, 1H, **8-H**), 7.74 (d, J = 2.1 Hz, 1H, **5-H**), 7.83 (d, J = 8 Hz, 1H, **4-H**), 8.02 (br s, 1H, **9-H**).

2-BENZYL-1,5-DIMETHYL-3,4-DIHYDRO-9-METHOXY-6H-PYRIDO [4,3-*b*] CARBAZOL-2-IUM CHLORIDE (**12a**)

Amide **11a** (0.77 g, 2 mmole) and $POCl_3$ (2 ml) were refluxed with stirring for 12 h in chloroform (10 mL). The yellow precipitate was then collected by filtration and washed with chloroform. The reaction product was recrystallized from methanol to give yellow crystals 0.79 g (97.6%), m. p. => 270°C. Elemental analysis for **12a** $C_{25}H_{25}N_2OCl$ x CH_3OH: M.w. 404.9 + 32.0; Calc.: C, 71.46%; H, 6.69%; N, 6.41%; Cl, 8.11%. Found: C, 71.5%; H, 6.6%; N, 6.35%; Cl, 8.1%. ^{1}H NMR (DMSO-d) δ: 2.54 (s, 3H, **1-CH_3**), 3.10 (s, 3H, **5-CH_3**), 3.19 (m, 2H, **4-CH_2**), 3.92 (s, 3H, **6-OCH_3**), 4.00 (t, 2H, **3-CH_2**), 5.42 (s, 2H, **-CH_2-**C_6H_5), 7.15 (dd, J = 8.8 Hz, J = 2.5 Hz, 1H, **8-H**), 7.49 (d, J = 8.0 Hz, 1H, **7-H**), 7.52-7.57 (m, 5H, -CH_2-**C_6H_5**), 7.95 (d, J = 2.4 Hz, 1H, **10-H**), 9.04 (s, 1H, **11-H**), 12.02 (s, 1H, **6-H**).

2-BENZYL-1-ETOXYCARBONYL-9-METHOXY-5-METHYL-3,4-DIHYDRO-6*H*-PYRIDO [4,3-*b*] CARBAZOL-2-IUM CHLORIDE (**12b**)

Amide **11b** (0.45 g, 1 mmole) and $POCl_3$ (5 mL) were refluxed with stirring for 24 h in toluene (50 mL). The final mixture was evaporated under reduced pressure to afford the residue, which was taken up into methanol (50 mL), and then the solution was evaporated to dryness. The solid residue was recrystalized from methylene dichloride to give dark--red crystals 0.4 g (87%), m. p. = 116-117°C. Elemental analysis for **12b** $C_{27}H_{27}N_2O_3Cl$: M. w. 463.0; Calc.: C, 70.05%; H, 5.88%; N, 6.05%; Cl, 7.66%. Found: C, 70.3%; H, 6.1%; N, 6.3%; Cl, 8.0%. ^{1}H NMR (DMSO-d) δ: 1.24 (t, 3H, $CH_2\mathbf{CH_3}$), 2.32 (s, 3H, **5-CH_3**), 3.43 (m, 2H, **4-CH_2**), 3.94 (s, 3H, **9-OCH_2**), 4.05 (m, 2H, **CH_2**CH_3), 4.28 (m, 2H, **3-CH_2**), 5.44 (s, 2H, **-CH_2-**C_6H_5), 7.05 (dd, J = 8.8 Hz, J = 2.6 Hz, 1H, **8-H**), 7.16-7.24 (m, 5H, -CH_2-**C_6H_5**), 7.26 (d, J = 8.8 Hz, 1H, **7-H**), 7.32 (d, J = 8.8 Hz, 1H, 7-H), 7.32 (d, J = 2.4 Hz, 1H, **10-H**), 7.76 (s, 1H, **11-H**), 11.37 (s, 1H, **6-H**).

2-BENZYL-1,5-DIMETHYL-9- METHOXY-1,2,3,4-TETRAHYDRO-6*H*-PYRIDO [4,3-*b*] CARBAZOLE (**13a**)

Intermediate iminium salt **12a** (1.62 g, 4 mmole) was dissolved in a mixture made of chloroform and methanol (25 mL: 40 mL), and it was reduced with an excess of $NaBH_4$ at room temperature. The stirred reaction mixture was left to stand for 2 h. Next, the solvent was evaporated, and reaction product **13a** was extracted with chloroform and recrystallized from ethanol to give 1.2 g (81%), m. p. 259-261°C. Elemental analysis for **13a** $C_{25}H_{26}N_2O$: M. w. 370.5; Calc.: C, 81.05%; H, 7.07%; N, 7.56%. Found: C, 81.2%; H, 6.9%; N, 7.3%. ^{1}H NMR (DMSO-d) δ: 1.44 (d, J = 6.4 Hz, 3H, **1-CH**), 2.42 (s, 3H, **5-CH**), 2.80 (m, 2H, **4-CH**), 3.18 (m, 2H, **3-CH**), 3.77 (s, 2H, **-CH_2-**C_6H_5), 3.85 (s, 3H, **9-OCH_3**), 4.10 (m, 1H, **1H**), 6.99 (dd, J = 8.7 Hz, J = 2.5 Hz, 1H, **8-H**), 7.32-7.40 (m, 6H, **7-H** + -CH_2-**C_6H_5**), 7.62 (d, J = 2.3 Hz, 1H, **10-H**), 7.68 (s, 1H, **11-H**), 10.73 (s, 1H, **6-H**).

1,5-DIMETHYL-9-METHOXY-6*H*-PYRIDO [4,3-*b*] CARBAZOLE (**4a**)

Preceding compound **13a** (0.74 g, 2 mmole) was refluxed in diphenyl ether (30 mL), in the presence of 10% palladium on charcoal (0.3 g) for 20 min (TLC monitoring). The catalyst was filtered off and the filtrate was cooled and diluted with hexane. The resulting precipitate was collected by filtration, washed with hexane and recrystallized from ethanol to give 0.514 g (93%) of 9-methoxyolivacine **4a**, in all aspects identical with the compound formerly describe (5, 11).

ETHYL 9-METHOXY-5-METHYL-6*H*-PYRIDO [4,3-*b*] CARBAZOLE-1-CARBOXYLATE (**4b**)

Intermediate iminium salt **12b** (0.926 g, 2 mmole) was dissolved in methanol (40 mL), and next reduced with an excess of $NaBH_4$ at room temperature. The stirred reaction mixture was left to stand for 4 h. Next, the solvent was evaporated, and the product of reduction **13b** was extracted with chloroform. After evaporation to dryness, the residue was dissolved and then refluxed in diphe-

Table 1. Cytostatic properties of L1210 mouse leukemia cells (IC_{50} values, μM) of compounds **12a** and **12b**, in comparison with elliptium (**3**), olivacine (**2**), and its derivative **4c**

L1210	Olivacine (2)[12]	Elliptium (3)[6]	4c	12a	12b
IC_{50}/μM	2.03	0.073[6]	2.49[6]	8.0	2.0

nyl ether (30 mL) in the presence of 10% palladium on charcoal (0.3 g) for 15 min (TLC monitoring). The catalyst was filtered off and the filtrate was cooled and diluted with hexane. The resulting precipitate was collected by filtration, washed with hexane, and recrystallized from ethyl acetate to give 0.325 g (48.6%) of expected compound **4b**. All its physical data were the same as those previously reported by us (6).

Biological test procedures

L1210 cells

Both new pyrido [4,3-*b*] carbazole derivatives (**12a**, **12b**) were evaluated *in vitro* against the L1210 mouse leukemia cell line (ATCC CCL210). L1210 cells were cultivated in Dulbecco's MEM (Gibco, Grand Island, U.S.A.) supplement with 10% fetal calf serum (FCS) at 37°C in air/CO_2 (5%) with a 100% relative humidity. Exponentially growing cells were seeded (10^5 cells/mL) in microwell plates (24 × 1 mL) and incubated for 24 h. After that time, cell density was approximately 3 × 10 cells/mL and increasing concentration of the tested compounds (from 10 to 10 M) was added in duplicate as DMSO solution, under a maximum volume of 5 μL/well. Control wells received 5 μL DMSO alone. After a further 24 h incubation, cells were numerated using a Coulter Counter ZM (Coultronics inc.). Growth inhibition releated to DMSO treated controls was measured from regression curves obtained with experimental points free of significant toxicity and was expressed as IC_{50}, the concentration that reduced by 50% the growth of treated cells as compared to controls. Data in Table 1 are the mean values of two independent experiments.

RESULTS AND DISCUSSION

The syntheses of aforementioned derivatives **4a** and **4b** were carried out by the sequence of reactions (Scheme 1).

The starting compound 2-[2-(benzylamino) ethyl]-6-methoxy-1-methylcabazole (**7**), which was previously described (6), after its transformation into 2-[2-(*N*-Acetyl-*N*-benzylamino) ethyl]-6-methoxy-1-methylcarbazole (**11a**) by the reaction with a mixture of acetic anhydride and pyridine (1: 1), and the following cyclization in boiling chloroform with $POCl_3$ gave iminium salt **12a**. Attempts on the direct oxidation with debenzylation of compound **12a** to be transformed into expected derivative **4a** ended up in failure. A number of degradation products of compound **12a** instead of expected derivative **4a**, were obtained. Taking into account all the facts described above, a procedure was conducted according to the Bessellievre and Husson method (9). At first, the reduction with $NaBH_4$ of dihydro derivative **12a** was carried out to obtain tetrahydro derivative 2-benzyl-9-methoxy-5-methyl-6*H*-pyrido [4,3-*b*] carbazole **13a**, and then product **13a** was aromatized by a simultaneous debenzylation to expected derivative **4a**, by the dehydrogenation over 10% palladium on charcoal in boiling diphenyl ether. The analogous sequence of the reactions was carried out, using diethyl oxalate as an acylating agent, for benzylamine derivative **7**, and respective amidoester **11b** was obtained. The last one (**11b**) was boiled in toluene with $POCl_3$ to give the product of cyclization as an iminium salt of pyrido [4,3-*b*] carbazole ring system **12b**. The reduction of **12b** to the intermediate **13b** was performed by using $NaBH_4$, and intermediate **13b** was immediately aromatized by dehydrogenation, with a simultaneous debenzylation over 10% palladium on charcoal in boiling diphenyl ether to give expected compound **4b**. Compounds **4a** and **4b** that have been obtained according to the method described above, exhibited the same physicochemical properties in comparison to those obtained and described in ref. 5, 6 (**4a** – 67% and **4b** – 31.4%). In this paper, we report on the synthesis of the said compounds with a good overall yield (**4a** – 72% and **4b** – 31%), and this procedure can be used as the alternative way for the synthesis of the title compounds.

New iminium salts of 2-benzyl-3,4-dihydro-9-methoxy-5-methyl-1-substituted-6*H*-pyrido [4,3-*b*] carbazole **12a-b**, which were subjected to a preliminary cytostatic activity screening, exhibited different biological properties, depending on the substituent type at position 1 in the main heterocyclic system; the respective data are shown in Table 1. In vitro, **12b** displays a cytostatic activity of the same or-

der of magnitude as compound **4c**, containing also the ester group at position 1, but in the case of derivative **12a** we observed the lowest activity. It can be seen that compound **12b** containing the methoxy-group at position 9, and compound **4c** were equal active, albeit the 9-hydroxy derivatives were more active than the corresponding 9-methoxy derivatives in most cases (6). Moreover, it can be seen that more bulky groups than the methyl group located at position 1 increase the cytostatic activity and that the presence of the benzyl group at position 2 does not significantly influence the activity.

The results presented in this paper do encourage us to further evaluation of some new iminium salts as the potent antiproliferative agents.

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Received: 15.11.2005