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SYNTHESIS OF NEW 1-PHENYL-6*H*-PYRIDO[4,3-b]CARBAZOLE DERIVATIVES WITH POTENTIAL CYTOSTATIC ACTIVITY

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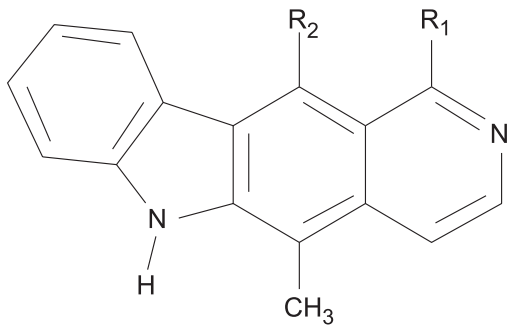
Abstract: A number of new 1-substituted-6*H*-pyrido[4,3-b]carbazole derivatives have been synthesized. Nine of the newly obtained compounds were subjected to preliminary *in vitro* cytostatic activity screening against murine leukemia (L1210), human lung cancer (A549) and human colon cancer (HT29) cell lines. One particular compound **6f** exhibited over 20 times better activity against L1210 tumor cell line than the reference ellipticine.

Keywords: olivacine, ellipticine, pyridocarbazole, cytostatic, L1210, A549, HT29

After the isolation of olivacine **1** and ellipticine **2** (Fig. 1), two plant alkaloids with antitumor properties (1–4), 6*H*-pyrido[4,3-*b*]carbazole became one of the most promising and intensively studied heterocyclic ring systems with both DNA intercalating and/or topoisomerase II inhibition properties (5, 6). Its planar structure was found to interact with DNA through intercalation often exhibiting a high DNA binding affinity The presence of electronegative ring nitrogen atoms distinguished ellipticine and its analogues from other simple intercalating molecules and enabled its protonation in physiological conditions. The positive charge of protonated molecule stabilized the binding of ellipticine derivatives to DNA nucleic acids, while the lipophilic uncharged moieties are responsible for penetration of membrane barriers (7).



1 $R_1 = CH_3$, $R_2 = H$

2 $R_1 = H$, $R_2 = CH_3$

Figure 1. Structure of natural alkaloids: olivacine **1** and ellipticine **2**

Despite much research on the synthetic ellipticine **2** and olivacine **1** analogues, there is still many unresolved questions of structure-activity relationships in this class of compounds. Until now, none of pyrido[4,3-*b*]carbazole derivatives have been commercialized but flexibility of this ring system for systematic modification has permitted the reliable application of rational drug design. Recently, two promising ellipticine **2** analogues NSC 69187 and NSC 338258 from the Developmental Therapeutics Program (DTP), at the National Cancer Institute (NCI).are intensively tested (8, 9).

As a results of our investigations on synthesis and biological properties of the 6*H*-pyrido[4,3-*b*]carbazole derivatives, we reported in our previous papers (10–15) the synthesis and activity of 1-phenyl and 1-pyridyl substituted pyrido[4,3-*b*]carbazoles. Some of these compounds demonstrated

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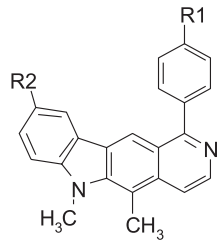


6c R_1=OCONHCH$_3$, R_2=OCONHCH$_3$
6d R_1=OSO$_2$CH$_3$, R_2=OSO$_2$CH$_3$
6c R_1=NH$_2$, R_2=OCH$_3$
6f R_1=NH$_2$, R_2=OH
6g R_1=NHCONHCH$_3$, R_2=OCONHCH$_3$
6h R_1=NHCONHCH$_3$, R_2=OCH$_3$
6i R_1=NHSO$_2$CH$_3$, R_2=OSO$_2$CH$_3$
6j R_1=NHSO$_2$CH$_3$, R_2=OCH$_3$

Figure 2. Structures of the newly obtained olivacine derivatives **6c-j**

strong cytostatic properties with broad spectrum of antitumor activity when tested *in vitro* on murine and human tumor cell lines. Basing on our previous results, we decided to insert the lipophilic phenyl group at 1 position of the main ring system with various OR or NHR substituents at 4' and 9 positions hoping to improve the bioavailability of drug molecule by facilitating its penetration through the cell membrane barriers.

All of the synthesized compounds were tested for their *in vitro* cytostatic activity against murine leukemia (L1210), human lung cancer (A549) and human colon cancer (HT29) cell lines. The newly obtained pyrido[4,3-*b*]carbazole derivatives (Fig. 2) were synthesized according to Scheme 1.

EXPERIMENTAL

Materials and Metods

Melting points were determined on a Köfler apparatus and are uncorrected; ^{1}H NMR spectra were recorded on a Bruker 300 at 300.14 MHz, using TMS as internal standard. Column chromatography was carried out on silica gel (Merck Kieselgel 100). All of the newly obtained compounds were analyzed for C, H, and N, and the analytical results were within ± 0.4% of theoretical values.

Chemistry

Syntheses of two compounds, namely 5,6-dimethyl-9-hydroxy-1-(4'-nitrophenyl)-6*H*-pyrido-[4,3-b]carbazole **6a** and 5,6-dimethyl-9-hydroxy-1-(4'-hydroxyphenyl)-6*H*-pyrido[4,3-b]-carbazole **6b**, have already been described (12). Compounds **4** and **6e** were obtained by heating of 9-methoxy-5-methyl-1-(4'-nitrophenyl)-6*H*-pyrido[4,3-*b*]carbazole **3a** or 5,6-dimethyl-9-methoxy-1-(4'-nitrophenyl)-6*H*-pyrido[4,3-*b*]carbazole **5a** with 10% palladium on charcoal in glacial acetic acid. Compound **6c** was obtained by reaction of 5,6-dimethyl-9-hydroxy-1-(4'-hydroxyphenyl)-6*H*-pyrido[4,3-*b*]carbazole **6b** with methyl isocyanate in dry chloroform. Both **6b** and **6f** were reacted with methanesulfonyl chloride in dry pyridine giving expected pyrido[4,3-*b*]carbazole derivatives **6d** and **6i,** respectively. Demethylation of amino derivative **6e** was performed by its heating in 48% hydrobromic acid and gave 5,6-dimethyl-9-hydroxy-1-(4'-aminophenyl)-6*H*-pyrido[4,3-*b*]carbazole **6f**. Compound **6f** was stirred in dry chloroform at room temperature with methyl isocyanate giving derivative **6g**.The same protocol for **6e** was used and led to the derivative **6h**. 5,6-Dimethyl-9-methoxy-1-[4'-(3-methylsulfamoyl)phenyl]-6*H*-pyrido[4,3-*b*]carbazole **6j** was prepared using reaction of **6e** with methanesulfonyl chloride in chloroform.

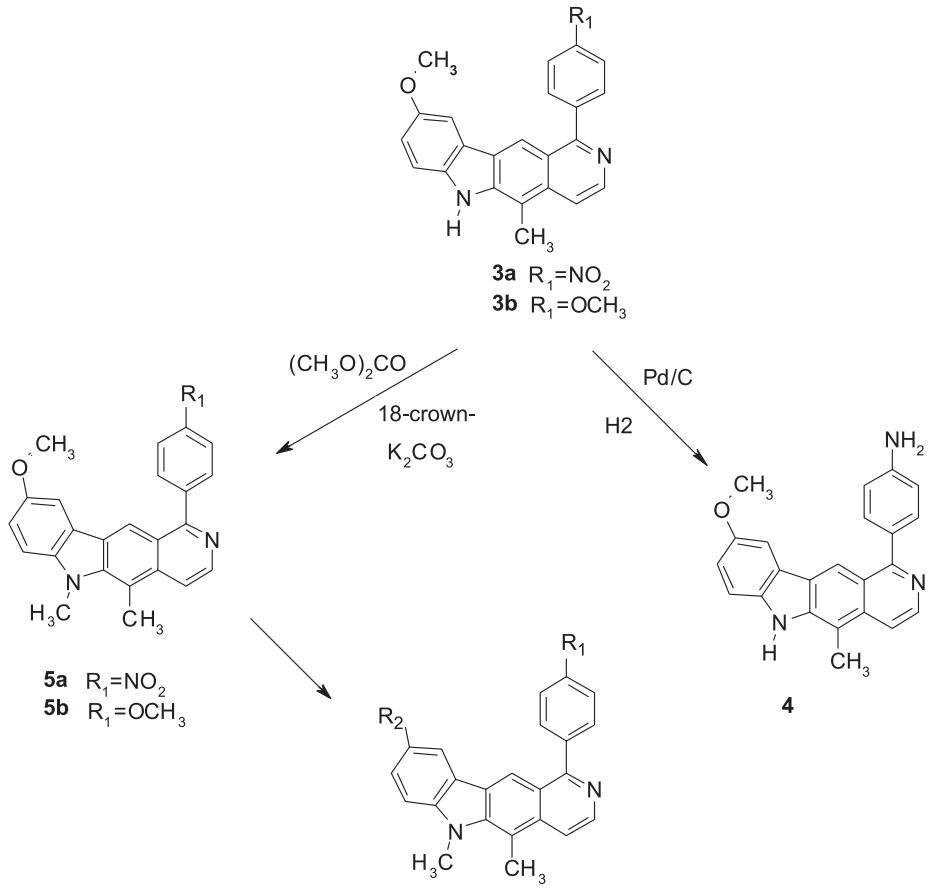
9-Methoxy-5-methyl-1-(4'-aminophenyl)-6H-pyrido[4,3-b]carbazole (**4**)

Compound **3a** (0.76 g, 2 mmol) was dissolved in 100 mL of glacial acetic acid and 10% palladium on charcoal (50 mg) was added. The mixture was warmed up to 50^{o}C and maintained at this temperature under hydrogen at normal pressure for 2 h. The catalyst was filtered off, the solvent was evaporated

to dryness, and the solid residue was purified by column chromatography on silica gel, eluent: methylene chloride : methanol, 9:1 v/v. By evaporating fractions containing only expected product the pure base was obtained. Yield: 45%, m.p.: 222°C. Analysis: calcd. for $C_{23}H_{19}N_3O$: C, 78.16 H, 5.42; N, 11.89%. Found: C, 78.35; H, 5.25; N, 11.70%. ^{1}H NMR (DMSO-d_6, δ, ppm): 2.81 (s, 3H, 5-CH_3), 3.85 (s, 3H, 9-OCH_3), 5.43 (s, 2H, 4'-NH_2), 6.75 (d, J = 8.3 Hz, 2H, phenyl-H), 7.11 (dd, $J_{8\text{-}7}$ = 8.7 Hz, $J_{8\text{-}10}$ = 2.4 Hz, 1H, 8-H), 7.43 (d, J = 8.3 Hz, 2H, phenyl-H), 7.49 (d, $J_{7\text{-}8}$ = 8.7 Hz, 1H, 7-H), 7.71 (d, $J_{10\text{-}8}$ = 2.4 Hz, 1H, 10-H), 7.80 (d, $J_{4\text{-}3}$ = 6.1 Hz, 1H, 4-H), 8.36 (d, $J_{3\text{-}4}$ = 6.1 Hz, 1H, 3-H), 8.74 (s, 1H, 11-H), 11.19 (s, 1H, 6-NH).

5,6-Dimethyl-9-methylcarbamoyloxy-1-[4'-(3-methylureilene)phenyl]-6H-pyrido[4,3-b]carbazole (**6c**)

A mixture of **6b** (0.17 g, 0.5 mmol), 4-dimethylaminopyridine (0.07 g, 0.55 mmol) and dry chloroform (100 mL) was stirred for 0.5 h and then



6a R_1=NO_2, R_2=OH
6b R_1=OH, R_2=OH
6c R_1=$OCONHCH_3$, R_2=$OCONHCH_3$
6d R_1=OSO_2CH_3, R_2=OSO_2CH_3
6e R_1=NH_2, R_2=OCH_3
6f R_1=NH_2, R_2=OH
6g R_1=$NHCONHCH_3$, R_2=$OCONHCH_3$
6h R_1=$NHCONHCH_3$, R_2=OCH_3
6i R_1=$NHSO_2CH_3$, R_2=OSO_2CH_3
6j R_1=$NHSO_2CH_3$, R_2=OCH_3

Scheme 1. Synthesis route of 1-phenylsubstituted-6*H*-pyrido[4,3-*b*]carbazole derivatives

methyl isocyanate (1 mL) was added. The mixture was stirred at room temperature for 24 h. After this time 10 mL of water was added, chloroform was separated and the mixture was extracted with 100 mL of chloroform. Organic layers were collected, dried and evaporated to dryness under reduced pressure. The solid residue was purified using column chromatography with silica gel, eluting with chloroform : methanol, 95:5 v/v. Yield: 45%, m.p.: 203–205°C. Analysis: calcd. for $C_{27}H_{24}N_4O_4$: C, 69.22; H, 5.16; N, 11.96%. Found: C, 69.05; H, 5.42; N, 11.67%. ^{1}H NMR (DMSO-d_6, δ, ppm): 2.73 (dd, 6H, 2×CH_3), 3.13 (s, 3H, 5-CH_3), 4.19 (s, 3H, 6-CH_3), 7.43 (m, 3H, phenyl-H + 8-H), 7.65 (d, $J_{7\text{-}8}$ = 8.8 Hz, 1H, 7-H), 7.77 (m, 3H, phenyl-H + 10-H), 8.09 (d, $J_{4\text{-}3}$ = 6,2 Hz, 1H, 4-H), 8.32 (m, 2H, 2×NH), 8.50 (d, $J_{3\text{-}4}$ = 6.2 Hz, 1H, 3-H), 8.59 (s, 1H, 11-H).

5,6-Dimethyl-9-methylsulfoxyl-1-[4'-(3-methylsulfoxyl)phenyl]-6H-pyrido[4,3-b]carbazole (**6d**)

A mixture of compound **6b** (0.17 g, 0.05 mmol), dry pyridine (10 mL) and methanesulfonyl chloride (1 mL) was stirred at room temperature for 1 h. After evaporation to dryness, the residue was taken up to water (50 mL), basified with sodium hydrogen carbonate, extracted with methylene chloride, and then dried over magnesium sulfate. After evaporation of solvent, the solid residue was purified by column chromatography on a silica gel by elution with methylene chloride : methanol, 9:1 v/v. Yield: 97%; m.p.: 269–271°C**.** Analysis: calcd. for: $C_{25}H_{22}N_2O_6S_2$: C, 58.81; H, 4.34; N, 5.49%. Found: C, 58.51; H, 4.69; N, 5.33%. ^{1}H NMR (DMSO-d_6, δ, ppm): 3.24 (s, 3H, 5-CH_3), 3.42 (s, 3H, 9-OSO_2CH_3), 3.57 (s, 3H, 4'-OSO_2CH_3), 4.31 (s, 3H, 6-CH_3), 7.67 (dd, $J_{8\text{-}7}$ = 8.8 Hz, $J_{8\text{-}10}$ = 2.3 Hz, 1H, 8-H), 7.75 (d, J = 8.5 Hz, 2H, phenyl-H), 7.86 (d, $J_{7\text{-}8}$ = 8.8 Hz, 1H, 7-H), 8.02 (d, J = 8.5 Hz, 2H, phenyl-H), 8.52 (m, 2H, 4-H + 10-H), 8.63 (d, $J_{3\text{-}4}$ = 7.1 Hz, 1H, 3-H), 8.99 (s, 1H, 11-H).

5,6-Dimethyl-9-methoxy-1-(4'-aminophenyl)-6H-pyrido[4,3-b]carbazole (**6e**)

This compound was synthesized using a similar procedure as described for compound **4** starting from **5a**. Yield 60%, m.p.: 117–118°C. Analysis: calcd. for: $C_{24}H_{21}N_3O$: C, 78.45; H, 5.76; N, 11.44%. Found: C, 78.70; H, 6.05; N, 11.16%; ^{1}H NMR (DMSO-d_6, δ, ppm): 3.06 (s, 3H, 5-CH_3), 3.85 (s, 3H, 9-OCH_3), 4.13 (s, 3H, 6-CH_3), 5.45 (s, 2H, 4'-NH_2), 6.76 (d, J = 8.2 Hz, 2H, phenyl-H), 7.16 (dd, $J_{8\text{-}7}$ = 8.8 Hz, $J_{8\text{-}10}$ = 2.5 Hz, 1H, 8-H), 7.47 (d, J = 8.2 Hz, 2H, phenyl-H), 7.52 (d, $J_{7\text{-}8}$ = 8.8 Hz, 1H, 7-H), 7.69 (d, $J_{10\text{-}8}$ = 2.5 Hz, 1H, 10-H), 7.87 (d, $J_{4\text{-}3}$ = 6.2 Hz, 1H, 4-H), 8.39 (d, $J_{3\text{-}4}$ = 6.0 Hz, 1H, 3-H), 8.75 (s, 1H, 11-H).

5,6-Dimethyl-9-hydroxy-1-(4'-aminophenyl)-6H-pyrido[4,3-b]carbazole (**6f**)

A mixture of compound **6e** (0.36 g, 1 mmol) and 48% hydrobromic acid (15 mL) was refluxed with stirring for 1 h. After evaporation to dryness, the residue was suspended in 50 mL of water. The resulting mixture was basified with conc. aq. ammonia and extracted with methylene chloride, then the organic layer was dried over magnesium sulfate. After evaporation of solvent, the solid residue was purified by chromatography on a silica gel column with methylene chloride : methanol, 9:1 v/v as eluent. Yield: 72%; m.p.: 225–226°C**.** Analysis: calcd. for: $C_{23}H_{19}N_3O$: C, 78.16; H, 5.42; N, 11.89%. Found: C, 77.83; H, 5.81; N, 11.73%. ^{1}H NMR (DMSO-d_6, δ, ppm): 3.06 (s, 3H, 5-CH_3), 4.11 (s, 3H, 6-CH_3), 5.50 (s, 2H, 4'- NH_2), 6.75 (d, J = 8.5 Hz, 2H, phenyl-H), 7.02 (dd, $J_{8\text{-}7}$ = 8.5 Hz, $J_{8\text{-}10}$ = 2.3 Hz, 1H, 8-H), 7.44 (m, 4H, 7-H + 10-H + phenyl-H), 7,90 (d, $J_{4\text{-}3}$ = 6.3 Hz, 1H, 4-H), 8.37 (d, $J_{3\text{-}4}$ = 6.3 Hz, 1H, 3-H), 8.61 (s, 1H, 11-H), 9.13 (s, 1H, 9-OH).

5,6-Dimethyl-9-methylcarbamoyloxy-1-[4'-(3-methylureilene)phenyl]-6H-pyrido[4,3-b]carbazole (**6g**)

This compound was synthesized using a similar procedure as described for compound **6c** starting from **6f**. Yield: 30%, m.p.: 240°C. Analysis: calcd. for $C_{27}H_{25}N_5O_3$: C, 69.36; H, 5.39; N, 14.98%. Found: C, 69.11; H, 5.68; N, 14.70%. ^{1}H NMR (DMSO-d_6, δ, ppm): 2.68 (m, 6H, 2× NH**C**$\mathbf{H_3}$), 3.11 (s, 3H, 5-CH_3), 4.18 (s, 3H, 6-CH_3), 6.21 (m, 1H, 4'-**NH**CH_3), 7.25 (dd, $J_{8\text{-}7}$ = 8.8 Hz, $J_{8\text{-}7}$ = 2.3 Hz, 1H, 8-H), 7.57 (d, $J_{7\text{-}8}$ = 8.5 Hz, 1H, 7-H), 7.61 (m, 4H, phenyl-H), 7.94 (d, $J_{10\text{-}8}$ = 2.3 Hz, 1H, 10-H), 7.98 (d, $J_{4\text{-}3}$ = 6,3 Hz, 1H, 4-H), 8.45 (d, $J_{3\text{-}4}$ = 6.3 Hz, 1H, 3-H), 8.70 (s, 1H, 11-H), 12.52 (s, 1H, 4'-NH).

5,6-Dimethyl-9-methoxy-1-[4'-(3-methylureilene)phenyl]-6H-pyrido[4,3-b]carbazole (**6h**)

This compound was synthesized using a similar procedure as described for compound **6c** starting from **6e**. Yield: 46%, m.p.: 262–263°C. Analysis: calcd. for $C_{26}H_{24}N_4O_2$: C, 73.57; H, 5.70; N, 13.20%. Found: C, 73.24; H, 6.08; N, 13.04%. ^{1}H NMR (DMSO-d_6, δ, ppm): 2.68 (d, J = 4.6 Hz, 3H, NH**C**$\mathbf{H_3}$), 3.09 (s, 3H, 5-CH_3), 3.83 (s, 3H, 9-OCH_3), 4.15 (s, 3H, 6-CH_3), 6.11 (m, 1H, **NH**-CH_3), 7.16 (dd, $J_{8\text{-}7}$ = 8.5 Hz, $J_{8\text{-}10}$ = 2.3 Hz, 1H, 8-H), 7.53

(d, $J_{7\text{-}8}$ = 8.8 Hz, 1H, 7-H), 7.62 (s, 4H, phenyl-H), 7.71 (d, $J_{10\text{-}8}$ = 2,6 Hz, 1H, 10-H), 7.96 (d, $J_{4\text{-}3}$ = 6.3 Hz, 1H, 4-H), 8.43 (d, $J_{3\text{-}4}$ = 6.3 Hz, 1H, 3-H), 8.70 (s, 1H, 11-H), 12.52 (s, 1H, 4'-NH).

5,6-Dimethyl-9-methylsulfoxyl-1-[4'-(3-methylsulfamoyl)phenyl]-6H-pyrido[4,3-b]carbazole (**6i**)

This compound was synthesized using a similar procedure as described for compound **6d** starting from **6f**.Yield 83%, m.p.: > 300°C. Analysis: calcd. for $C_{25}H_{23}N_3O_5S_2$: C, 58.92; H, 4.55; N, 8.25%. Found: C, 59.11; H, 4.72; N, 8.06%. ^{1}H NMR (DMSO-d_6, δ, ppm): 2.30 (s, 3H, 5-CH_3), 3.21 (s, 6H, 9-SO_2CH_3 + 4'-SO_2CH_3), 4.30 (s, 3H, 6-CH_3), 7.54 (d, J = 8.5 Hz, 2H, phenyl-H), 7.65 (dd, $J_{8\text{-}7}$ = 8.8 Hz, $J_{8\text{-}10}$ = 2.3 Hz, 1H, 8-H), 7.87 (m, 3H, phenyl-H + 7-H), 8.47 (d, $J_{4\text{-}3}$ = 6.8 Hz, 1H, 4-H), 8.51 (d, $J_{10\text{-}8}$ = 2.3 Hz, 1H, 10-H), 8.58 (d, $J_{3\text{-}4}$ = 7.2 Hz, 1H, 3-H), 9.02 (s, 1H, 11-H), 10.46 (s, 1H, 4'-NH).

5,6-Dimethyl-9-methoxy-1-[4'-(3-methylsulfamoyl)phenyl]-6H-pyrido[4,3-b]carbazole (**6j**)

Compound **6e** (0.18 g, 0.5 mmol) and 4-dimethylaminopyridine (0.07 g, 0.55 mmol) were dissolved in chloroform (50 mL). Then, methanesulfonyl chloride (1 mL) was added after 30 min with stirring and the reaction mixture was maintained at room temperature for 24 h. After this time, the solvent was evaporated under reduced pressure and the solid residue was purified using column chromatography with neutral alumina Grad II and dichloromethane as eluent. Yield: 41%, m.p.: 272–273°C. Analysis: calcd. for $C_{25}H_{23}N_3O_3S$: C, 67.40; H, 5.20; N, 9.43%. Found: C, 67.23; H, 5.47; N, 9.17%. ^{1}H NMR (DMSO-d_6, δ, ppm): 3.10 (s, 3H, 5-CH_3), 3.14 (s, 3H, SO_2CH_3), 3.83 (s, 3H, 9-OCH_3), 4.15 (s, 3H, 6-CH_3), 7.17 (dd, $J_{8\text{-}7}$ = 8.8 Hz, $J_{8\text{-}10}$ = 2.6 Hz, 1H, 8-H), 7.44 (d, J = 8.6 Hz, 2H, phenyl-H), 7.53 (d, $J_{7\text{-}8}$ = 8.8 Hz, 1H, 7-H), 7.71 (d, J = 8.6 Hz, 2H, phenyl-H), 7.77 (d, $J_{10\text{-}8}$ = 2.3 Hz, 1H, 10-H), 8.00 (d, $J_{4\text{-}3}$ = 6.3 Hz, 1H, 4-H), 8.45 (d, $J_{3\text{-}4}$ = 6.3 Hz, 1H, 3-H), 8.68 (s, 1H, 11-H), 10.05 (s, 1H, 4'-NH).

Biological test procedures

Test solutions of nine new pyrido[4,3-*b*]carbazole derivatives **4**, **6c-6j** (1 mg/mL) were prepared *ex tempore* for each test by dissolving them in 100 µL of DMSO + 900 µL of culture medium. Then, the solutions were diluted in culture medium to reach the final concentrations of 100 to 0.1 mg/mL.

Cell lines

The L1210 (murine leukemia), A549 (human lung cancer) and HT29 (human colon cancer) cell lines were used. Our lines were cultured in the Cell Culture Collection of the Department of Tumor Immunology, Institute of Immunology and Experimental Therapy, Wrocław, Poland. The L1210 were cultivated in the RPMI 1640 GlutaMax medium supplemented with 10% fetal calf serum (FCS), glutamine (2 mM), sodium pyruvate (1 mM), glucose (4.5 g/L), penicillin (100 U/mL), and streptomycin (100 µg/mL). The A549 and HT-29 cells

Table 1. Cell growth inhibition on L1210 (murine leukemia), A549 (human lung cancer) and HT29 (human colon cancer) cell lines. IC_{50} values [µM] ± SD of compounds **4, 6c-6j** compared with ellipticine.

Compound	IC_{50}[µM] ± SD		
	L1210	A549	HT29
4	1.35 ± 0.65	0.933 ± 0.02	2.3 ± 0.52
6c	5.02 ± 1.50	3.20 ± 1.06	4.7 ± 0.80
6d	inactive	–	–
6e	3.82 ± 0.52	0.65 ± 0.35	2.7 ± 0.46
6f	0.096 ± 0.031	0.51 ± 0.22	6.5 ± 1.2
6g	20.8±12.1	–	–
6h	4.76 ± 0.30	1.32 ± 0.53	2.3 ± 0.21
6i	inactive	–	–
6j	8.04 ± 0.82	1.10 ± 0.22	6.4 ± 1.01
Ellipticine	2.43 ± 0.73	0.85 ± 0.04	1.66 ± 0.04

were cultivated in the RPMI 1640 opti-MEM medium supplemented with 5% fetal calf serum (FCS), glutamine (2 mM), penicillin (100 U/mL), and streptomycin (100 µg/mL). Additionally, the HT-29 cell line medium was prepared using 1 mM sodium pyruvate addiction. The cell cultures were maintained at 37°C in a humid atmosphere containing 5% CO_2.

MTT and SRB

The MTT (for L1210 murine leukemia) or SRB (for A549 human lung cancer and HT29 human colon cancer) methods were used as described by Skehan et al. (16). The cytostatic assays were performed after 96-hour exposure of the cultured cells to varying concentrations of the tested agents. Each experiment was repeated three times. IC_{50} values were determined by the concentration of the compound required to inhibit cells proliferation in 50% taking into account the cytostatic properties of DMSO used for dissolving the compounds tested.

RESULTS AND DISCUSSION

Nine of the newly obtained compounds (**4**, **6c-j**) were subjected to preliminary *in vitro* cytostatic activity screening against murine leukemia L1210 cell line. Three of them were almost inactive (**6d, 6g, 6i**) and were excluded from further investigations. Six compounds with significant activity were additionally tested *in vitro* for their cytostatic properties on two human tumor cell lines (A549 and HT29) in comparison with reference – ellipticine. The results of biological screening are assembled in Table 1. Considering compounds with sterically large substituents at 4' and 9 positions (**6c, 6d, 6g, 6i**) only **6c** showed significant but not strong antiproliferative properties. On the other hand, all compounds with relatively small substituents at 4' and 9 positions (**4, 6e, 6f**) showed biological activity only slightly worse or comparable with reference ellipticine except of **6f,** which was about 20 times more active on murine leukemia L1210 cell line than the reference. Compounds **6h** and **6j** with small methoxy group at 9 position and a large substituent at 4' position showed moderate antiproliferative properties on all of the tumor cell lines tested.

The results of the *in vitro* biological study indicated that the presence of large methylcarbamoyl or methylsulfonyl moieties at 4' and/or 9 positions of 1-phenyl-6*H*-pyrido[4,3-b]carbazole ring system didn't significantly increase the cytostatic properties of synthesized compounds in comparison with mother compound **6f**. It is specially worth to notice that the substitution of 9 position with methylsulfoxyl group led to complete loss of activity (compounds **6d**, **6i**).

Compounds **4**, **6e 6f** and **6h** were approximately as active as reference ellipticine on human tumor cell line tested. Compound **6f** with amino group at 4' position was over 20 times more active against L1210 leukemia cell line in comparison with ellipticine and should be considered as the most active compound of the described series.

It is also possible to compare the activities of presented derivatives with recently reported closely related compounds (15) with methoxy group at 2' position because both groups of compounds were tested on the same tumor cell line A549 (non-small cell lung cancer). This comparison may be briefly summarized that the presence of methoxy group at 2' position showed no significant influence on antitumor properties of 1-phenyl substituted olivacine derivatives.

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