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SYNTHESIS AND STRUCTURE-ACTIVITY RELATIONSHIP ANALYSIS OF NEW OLIVACINE DERIVATIVES

BEATA TYLIŃSKA[1]*, RYSZARD JASZTOLD-HOWORKO[1], HENRYK MASTALARZ[1], DAGMARA KŁOPOTOWSKA[2], BEATA FILIP[2] and JOANNA WIETRZYK[2]

[1] Wrocław Medical University, Faculty of Pharmacy, Department of Organic Chemistry, Grodzka 9, 50-137 Wrocław, Poland
[2] Institute of Immunology and Experimental Therapy, Polish Academy of Sciences, Rudolfa Weigla 12, 53-114 Wrocław, Poland

Abstract: The number of 3'- and 4'-substituted 1-phenyl-6H-pyrido[4,3-*b*]carbazole derivatives have been synthesized and tested biologically. Eleven of the newly obtained compounds were subjected to the preliminary cytostatic screening for their activity against L1210 (murine leukemia), A498 (human kidney cancer), A549 (human lung cancer) and HT29 (human colon cancer) cell lines. Eight of tested derivatives exhibited significant biological activities, which only weakly depended on side chain length and position of the substituent.

Keywords: olivacine, pyridocarbazole, cytostatic, L1210, A498, A549, HT29

Olivacine **1** (Fig. 1) was firstly isolated from *Aspidosperma olivaceum* Müll. Arg. by J. Schmutz and A.F. Huzicker in 1958 (1). During further investigations this natural alkaloid exhibited marked antineoplastic activity (2). Its mechanism of action is considered to be mainly based on DNA intercalation and/or inhibition of topoisomerase II (3, 4).

Many structural analogues of olivacine **1** have been synthesized recently and their antitumor properties have been extensively studied. These investigations resulted in development of several drugs like *pazaelliptine* (5, 6) or *retelliptine* (7, 8). One of the pyrido[4,3-*b*]carbazole derivatives (S 16020-2) demonstrated a broad spectrum of antitumor activity on murine (P388 leukemia, Lewis lung carcinoma, B16 melanoma, M5076 sarcoma) and human (colon, breast, ovary, lung) tumor cell lines (9–17). Some other olivacine analogues, namely 1-pyridylsubstituted pyrido[4,3-*b*]carbazole derivatives have also shown strong cytostatic activity when tested *in vitro* (18–20). In earlier paper we have described the synthesis and cytostatic activity of related olivacine derivatives**.** Two of our compounds have shown strong cytostatic activity on human lung cancer (A549) (**6a** IC_{50}= 2.37 μM, **6b,** IC_{50}= 1.76 μM**)** (Fig. 2) (19).

These results encouraged us to synthesize and investigate a new series of 1-phenyl-6*H*-pyrido[4,3-*b*]carbazole derivatives (Fig. 3). We have decided to

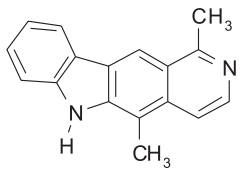

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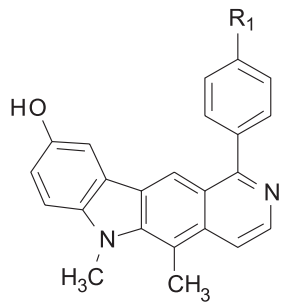
Figure 1. Structure of the natural alkaloid olivacine **1**



6a R_1= NO_2
6b R_1= OH

Figure 2. Structures of 1-phenylsubstituted pyrido[4,3-*b*]carbazole derivatives **6a-b**

* e-mail: diana@chorg.am.wroc.pl; fax (+48 71) 784 0341

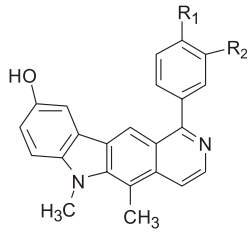


Figure 3. General structure of the newly obtained olivacine erivatives

modify the structure of main ring system only at 4' and 3' positions. Eleven of the newly obtained compounds were subjected to preliminary *in vitro* cytostatic activity screening against murine leukemia (L1210), human kidney cancer (A498), human lung cancer (A549) and human colon cancer (HT29) cell lines.

All of the new pyrido[4,3-*b*]carbazole derivatives (Fig. 3) were obtained according to Scheme 1.

EXPERIMENTAL

Melting points were determined on a Köfler apparatus and were uncorrected. ^{1}H NMR spectra were recorded on a Tesla BS 587 A at 80 MHz or on a Bruker 300 at 300.14 MHz (Bruker, Rheinstetten, Germany), using TMS as an internal standard. Column chromatography was carried out on silica gel (Merck Kieselgel 100; Merck, Darmstadt, Germany). All of the newly obtained compounds were analyzed for C, H, and N and the analytical results were within ± 0.4% of the theoretical values.

Synthetic chemistry

The starting compound 2-(6-methoxy-1-methyl-9*H*-carbazol-2-yl)ethylamine **4** was prepared according to a previously described procedure (7). Compounds **5** and **6** were obtained by heating of terephthalic acid monomethyl ester or isophthalic acid monomethyl ester with amine **4**. Cyclization of the resulting amides **5** or **6** with phosphorus oxychloride in boiling toluene gave derivatives **7** or **8**, which were aromatized to compounds **9** or **10** by dehydrogenation over 10% palladium on charcoal in boiling diphenyl ether. Esters **11** and **12** were obtained using dry potassium carbonate, dimethyl carbonate and 18-crown-6 (or Adogen 464), which reacted with *N,N*-dimethylethane-1,2-diamine, *N,N*-dimethylpropane-1,3-diamine or 2-amino-2-methyl-1-propanol giving amides **13**, **14**, **15**, **16** or **17,** respectively. Compounds **11** or **12** were successfully 9-*O*-demethylated by reaction with boron tribromide at –70˚C to afford derivatives **18**, **20** or **21**.

Amides **19**, **22**, **23**, **24** were obtained by reaction of the mother compounds **18**, **20** or **21** with *N,N*-dimethylethane-1,2-diamine or *N,N*-dimethylpropane-1,3-diamine at room temperature.

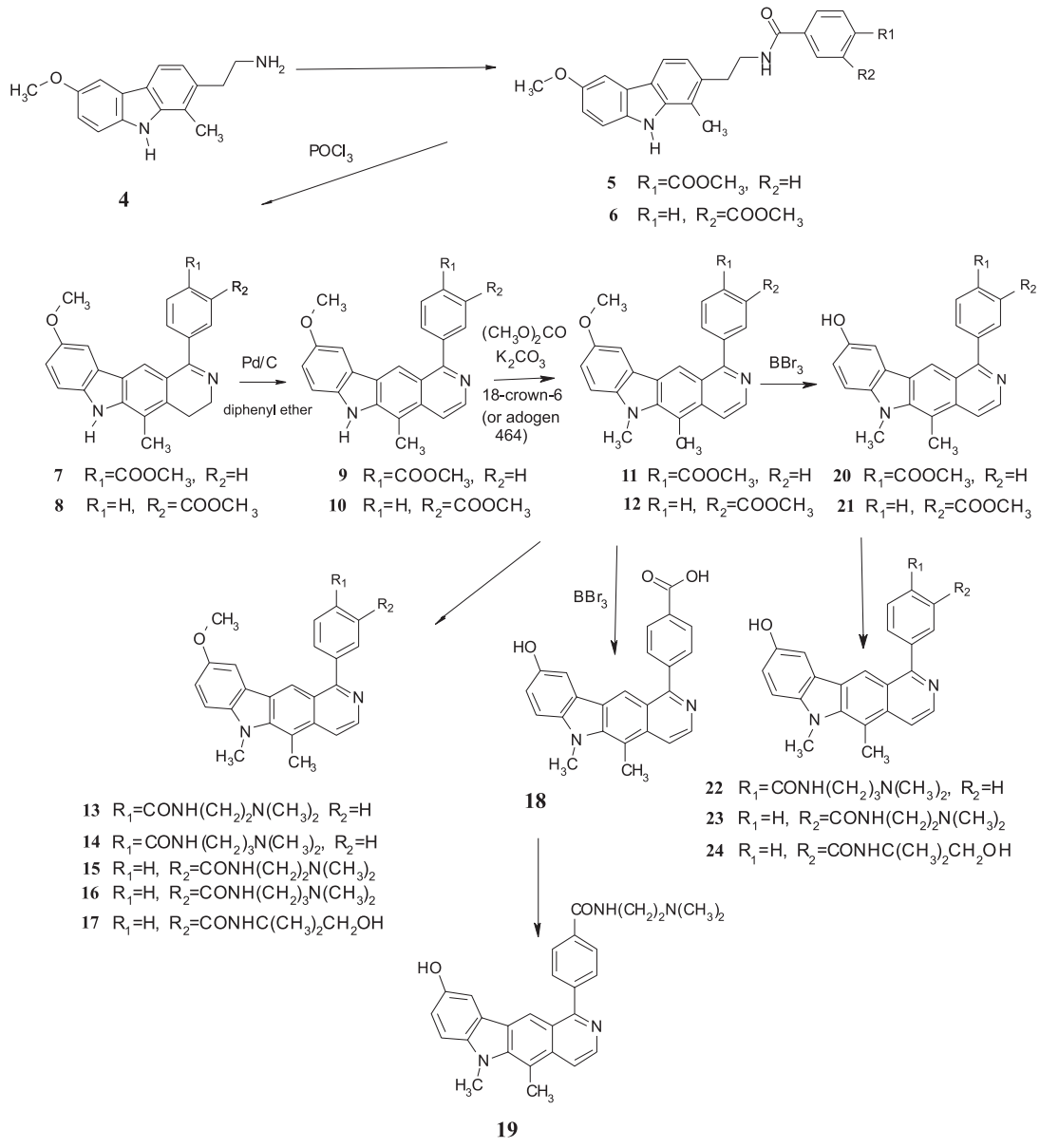
N-[2-(6-Methoxy-1-methyl-9H-carbazol-2-yl)ethyl]-terephthalamic acid methyl ester (**5**) and
N-[2-(6-methoxy-1-methyl-9H-carbazol-2-yl)ethyl]-isophthalamic acid methyl ester (**6**)

Triethylamine (0.5 g, 5 mmol) was added to terephthalic acid monomethyl ester (or isophthalic acid monomethyl ester) (0.79 g, 4.4 mmol) dissolved in dry tetrahydrofuran (THF, 100 mL). After cooling to –10^{o}C, a solution of ethyl chloroformate (0.54 g, 5 mmol) in dry THF (10 mL) was added to the resulting mixture with stirring. The mixture was stirred for a further 30 min and then a solution of 2-(6-methoxy-1-methyl-9*H*-carbazol-2-yl)ethylamine **4** (1.01 g, 4 mmol) in dry THF (100 mL) was added dropwise at –10^{o}C. The resulting mixture was left for 24 h to reach room temperature with stirring and then the precipitate was collected by filtration and the filtrate was evaporated to dryness. The resulting residue was suspended in water (50 mL), alkalized with conc. aq. ammonia, extracted with 200 mL of CH_2Cl_2, and the extract was dried over magnesium sulfate. Evaporation of the solvent provided a solid residue, which was recrystallized from ethanol.

5: Yield: 0.87 g (48%), m.p. 239–240^{o}C. ^{1}H NMR (DMSO-d_6, δ, ppm): 2.52 (s, 3H, 1-CH_3), 3.00 (m, 2H, β-CH_2), 3.47 (m, 2H, α-CH_2), 3.81 (s, 3H, 6-OCH_3), 3.86 (s, 3H, 4'-$COOCH_3$), 6.96 (m, 2H, 7-H, 3-H), 7.36 (d, $J_{8\text{-}7}$ = 8.7 Hz, 1H, 8-H), 7.57 (d, $J_{5\text{-}7}$ = 2.4 Hz, 1H, 5-H), 7.81 (d, $J_{4\text{-}3}$ = 7.9 Hz, 1H, 4-H), 7.98 (m, 4H, phenyl-H), 8.48 (m, 1H, -NHCO), 10.83 (s, 1H, 9-NH). Analysis: calcd. for $C_{25}H_{24}N_2O_4$: C 72.10, H 5.81, N 6.73%; found: C 71.82, H, 6.08, N 6.57%.

6: Yield: 1.0 g (55%), m.p. 179–180^{o}C. ^{1}H NMR (DMSO-d_6, δ, ppm): 2.52 (s, 3H, 1-CH_3), 3.00 (m, 2H, β-CH_2), 3.47 (m, 2H, α-CH_2), 3.81 (s, 3H, 6-OCH_3), 3.87 (s, 3H, 3'-$COOCH_3$), 6.96 (m, 2H, 7-H, 3-H), 7.35 (d, $J_{8\text{-}7}$ = 8.7 Hz, 1H, 8-H), 7.58 (d, $J_{5\text{-}7}$ = 2.4 Hz, 1H, 5-H), 7.63 (m, 1H, 5'-H), 7.82 (d, $J_{4\text{-}3}$ = 7.9 Hz, 1H, 4-H), 8.09 (m, 2H, 4'-H, 6'-H), 8.43 (s, 1H, 2'-H), 8.88 (m, 1H, -NHCO), 10.84 (s, 1H, 9-NH). Analysis: calcd. for $C_{25}H_{24}N_2O_4$: C 72.10, H 5.81, N 6.73%; found: C 71.93, H 6.07, N 6.47%.

9-Methoxy-5-methyl-1-(4-methoxycarbonylphenyl)-3,4-dihydro-6H-pyrido[4,3-b]carbazole (**7**) and
9-methoxy-5-methyl-1-(3-methoxycarbonylphenyl)-3,4-dihydro-6H-pyrido[4,3-b]carbazole (**8**)



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

The amide **5** (0.86 g, 2 mmol) (or **6** 0.89 g 2 mmol) was dissolved in boiling toluene (120 mL) and then treated dropwise with $POCl_3$ (12 mL). The reflux was continued for 5 h and evaporation under reduced pressure afforded a residue which was put into water (100 mL), alkalized with $NaHCO_3$, and extracted with CH_2Cl_2. The extract was dried over magnesium sulfate. After evaporation of the solvent, the solid residue was purified by column chromatography on silica gel and eluted with CH_2Cl_2 : CH_3OH (95 : 5, v/v). The pure base was obtained by evaporating fractions containing the expected product.

7: Yield: 0.38 g (48%), m.p. 260–261°C. ^{1}H NMR (DMSO-d_6, δ, ppm): 2.51 (s, 3H, 5-CH_3), 2.84 (m, 2H, 4-CH_2), 3.72 (m, 5H, 9-OCH_3, 3-CH_2), 3.89 (s, 3H, 4'-$COOCH_3$), 6.97 (dd, $J_{8\text{-}7}$ = 8.8 Hz, $J_{8\text{-}10}$ =

2.5 Hz, 1H, 8-H), 7.40 (d, $J_{7\text{-}8}$ = 8.8 Hz, 1H, 7-H), 7.53 (d, $J_{10\text{-}8}$ = 2.4 Hz, 1H, 10-H), 7.71 (d, J = 8.2 Hz, 2H, 6'-H, 2'-H), 7.74 (s, 1H, 11-H), 8.05 (d, J = 8.2 Hz, 2H, 5'-H, 3'-H), 11.25 (s, 1H, 6H). Analysis: calcd. for $C_{25}H_{22}N_2O_3$: C 75.36, H 5.57, N 7.03%; found: C 75.03, H 5.95, N, 6.87%.

8: Yield: 0.51 g (65%), m.p. 230–231°C. ^{1}H NMR (DMSO-d_6, δ, ppm): 2.51 (s, 3H, 5-CH_3), 2.84 (m, 2H, 4-CH_2), 3.72 (m, 5H, 9-OCH_3, 3-CH_2), 3.85 (s, 3H, 3'-$COOCH_3$), 6.98 (dd, $J_{8\text{-}7}$ = 8.8 Hz, $J_{8\text{-}10}$ = 2.5 Hz, 1H, 8-H), 7.39 (d, $J_{7\text{-}8}$ = 8.8 Hz, 1H, 7-H), 7.53 (d, $J_{10\text{-}8}$ = 2.4 Hz, 1H, 10-H), 7.63 (m, 1H, 5'-H), 7.74 (s, 1H, 11-H), 7.82 (m, 1H, 6'-H,), 8.07 (m, 1H, 4'-H), 8.16 (m, 1H, 2'-H), 11.20 (s, 1H, 6H). Analysis: calcd. for $C_{25}H_{22}N_2O_3$: C 75.36, H 5.57, N 7.03%; found: C 75.05, H 5.95, N 6.85%.

9-Methoxy-5-methyl-1-(4-methoxycarbonylphenyl)-6H-pyrido[4,3-b]carbazole (**9**) and 9-methoxy-5-methyl-1-(3-methoxycarbonylphenyl-6H-pyrido[4,3-b]carbazole (**10**)

Compound **7** (0.79 g, 2 mmol) or **8** (0.79 g, 2 mmol) was refluxed in diphenyl ether (50 mL) in the presence of 10% palladium on charcoal (0.10 g) for 0.5 h. The catalyst was filtered off and the filtrate was cooled and diluted with 100 mL of *n*-hexane. The resulting precipitate was collected and washed with *n*-hexane. The filtrate was then extracted with 20 mL of 5% HCl solution, then the water layer was separated and alkalized with conc. aq. ammonia, extracted with 200 mL of CH_2Cl_2, and organic layer was dried over magnesium sulfate. After evaporation of the solvent both precipitates were combined and purified by chromatography on silica gel column with CH_2Cl_2 : CH_3OH mixture (95 : 5, v/v) as an eluent. The pure base was obtained by evaporation of the the fractions containing the expected product.

9: Yield: 0.60 g (76%), m.p. 256–257°C. ^{1}H NMR (DMSO-d_6, δ, ppm): 2.86 (s, 3H, 5-CH_3), 3.81 (m, 3H, 9-OCH_3), 3.93 (s, 3H, 4'-$COOCH_3$), 7.12 (dd, $J_{8\text{-}7}$ = 8.8 Hz, $J_{8\text{-}10}$ = 2.5 Hz, 1H, 8-H), 7.44 (d, $J_{7\text{-}8}$ = 8.8 Hz, 1H, 7-H), 7.77 (d, $J_{10\text{-}8}$ = 2.4 Hz, 1H, 10-H), 7.87 (d, J = 8.2 Hz, 2H, 6'-H, 2'-H), 8.00 (d, $J_{4\text{-}3}$ = 6.1 Hz, 1H, 4-H), 8.18 (d, J = 8.2 Hz, 2H, 5'-H, 3'-H), 8.48 (d, $J_{3\text{-}4}$ = 6.1 Hz, 1H, 3-H), 8.60 (s, 1H, 11-H), 11.28 (s, 1H, 6-NH). Analysis: calcd. for $C_{25}H_{20}N_2O_3$: C 75.74, H 5.08, N 7.07%; found: C 75.49, H 5.37, N, 6.79%.

10: Yield: 0.67 g (85%), m.p. 253°C. ^{1}H NMR (DMSO-d_6, δ, ppm): 2.86 (s, 3H, 5-CH_3), 3.80 (s, 3H, 9-OCH_3), 3.88 (s, 3H, 3'-$COOCH_3$), 7.12 (dd, $J_{8\text{-}7}$ = 8.8 Hz, $J_{8\text{-}10}$ = 2.5 Hz, 1H, 8-H), 7.44 (d, $J_{7\text{-}8}$ = 8.8 Hz, 1H, 7-H), 7.75 (m, 2H, 10-H, 5'-H), 7.99 (m, 2H, 6'-H, 4-H), 8.15 (m, 1H, 4'-H), 8.27 (m, 1H, 2'-H), 8.47 (d, $J_{3\text{-}4}$ = 6.1 Hz, 1H, 3-H), 8.57 (s, 1H, 11-H), 11.27 (s, 1H, 6-NH). Analysis: calcd. for $C_{25}H_{20}N_2O_3$: C 75.74, H 5.08, N 7.07%; found: C 75.51, H 5.35, N 6.78%.

5,6-Dimethyl-9-methoxy-1-(4-methoxycarbonylphenyl)-6H-pyrido[4,3-b]carbazole (**11**) and 5,6-dimethyl-9-methoxy-1-(3-methoxycarbonylphenyl)-6H-pyrido[4,3-b]carbazole (**12**)

A mixture of compound **9** (0.79 g, 2 mmol) or (**10** 0.79 g, 2 mmol), dry potassium carbonate (0.5 g), dimethyl carbonate (15 mL), DMF (2 mL), and the appropriate transfer phase catalyst (0.02 g of 18-crown-6 or 0.14 g of Adogen 464) was refluxed with stirring for 24 h (or 3 h when Adogen 464 was used). After evaporation to dryness, the residue was taken up to water, extracted with CH_2Cl_2 and extract was dried over magnesium sulfate. Evaporation of the solvent provided a solid residue, which was purified by chromatography on a silica gel column and eluted with CH_2Cl_2 : C_2H_5OH (90 : 10, v/v) .

11: Yield: 0.45 g (55%), m.p. 220°C. ^{1}H NMR (DMSO-d_6, δ, ppm): 3.11 (s, 3H, 5-CH_3), 3.81 (m, 3H, 9-OCH_3), 3.93 (s, 3H, 4'-$COOCH_3$), 4.16 (s, 3H, 6-CH_3), 7.17 (dd, $J_{8\text{-}7}$ = 8.8 Hz, $J_{8\text{-}10}$ = 2.5 Hz, 1H, 8-H), 7.54 (d, $J_{7\text{-}8}$ = 8.8 Hz, 1H, 7-H), 7.76 (d, $J_{10\text{-}8}$ = 2.5 Hz, 1H, 10-H), 7.87 (d, J = 8.2 Hz, 2H, 6'-H, 2'-H), 8.08 (d, $J_{4\text{-}3}$ = 6.2 Hz, 1H, 4-H), 8.18 (d, J = 8.2 Hz, 2H, 5'-H, 3'-H), 8.49 (d, $J_{3\text{-}4}$ = 6.2 Hz, 1H, 3-H), 8.61 (s, 1H, 11-H). Analysis: calcd. for $C_{26}H_{22}N_2O_3$: C 76.08, H 5.40, N 6.82%; found: C 75.89, H 5.57, N 6.63%.

12: Yield: 0.70 g (86%), m.p. 214°C. ^{1}H NMR (DMSO-d_6, δ, ppm): 3.11 (s, 3H, 5-CH_3), 3.80 (s, 3H, 9-OCH_3), 3.88 (s, 3H, 3'-$COOCH_3$), 4.15 (s, 3H, 6-CH_3), 7.17 (dd, $J_{8\text{-}7}$ = 8.8 Hz, $J_{8\text{-}10}$ = 2.5 Hz, 1H, 8-H), 7.53 (d, $J_{7\text{-}8}$ = 8.8 Hz, 1H, 7-H), 7.75 (m, 2H, 10-H, 5'-H), 8.00 (m, 1H, 6'-H), 8.06 (d, $J_{4\text{-}3}$ = 6.2 Hz, 1H, 4-H), 8.15 (m, 1H, 4'-H), 8.27 (m, 1H, 2'-H), 8.48 (d, $J_{3\text{-}4}$ = 6.2 Hz, 1H, 3-H), 8.58 (s, 1H, 11-H). Analysis: calcd. for $C_{26}H_{22}N_2O_3$: C 76.08, H 5.40, N 6.82%; found: C 75.93, H 5.55, N 6.61%.

General procedure for the synthesis of amides **13–17, 22–24**

A mixture of compound **11** (or **12, 20, 21,** respectively), (0.5 mmol), *N,N*-dimethylethylenediamine (10 mL) (or *N,N*-dimethyl-1,3-propanediamine (10 mL), or 2-amino-2-methyl-1-propanol (10 mL), respectively) and a few drops of DMF was stirred in nitrogen atmosphere at normal pressure for 2 h. After evaporation to dryness, the residue was purified by column chromatography on silica gel column and eluted with CH_2Cl_2 : CH_3OH (95 : 5, v/v).

5,6-Dimethyl-9-methoxy-1-{4-[N-[2-(N,N-dimethylamino)ethyl]carbamoyl]phenyl}-6H-pyrido[4,3-b]carbazole (**13**)

13: Yield 0.066 g (29%), m.p. 110–111°C. ^{1}H NMR (DMSO-d_6, δ, ppm): 2.48 (s, 6H, -N(CH_3)$_2$), 2.71 (m, 2H, β-CH_2), 3.03 (s, 3H, 5-CH_3), 3.30 (m, 2H, α-CH_2), 3.81 (s, 3H, 9-OCH_3), 4.15 (s, 3H, 6-CH_3), 7.17 (dd, $J_{8\text{-}10}$ = 2.4 Hz, $J_{8\text{-}7}$ = 8.8 Hz, 1H, 8-H), 7.54 (d, 1H, $J_{7\text{-}8}$ = 8.9 Hz, 7-H), 7.69 (d, $J_{10\text{-}8}$ = 2.3 Hz, 1H, 10-H), 7.82 (d, J = 8.1 Hz, 2H, 6'-H, 2'-H), 8.06 (d, $J_{3\text{-}4}$ = 6.2 Hz, 1H, 4-H), 8.10 (d, J = 8.1 Hz, 2H, 5'-H, 3'-H), 8.48 (d, $J_{3\text{-}4}$ = 6.2 Hz, 1H, 3-H), 8.60 (s, 1H, 11-H), 8.84 (m, 1H, -CO**NH**CH_2). Analysis: calcd. for $C_{29}H_{30}N_4O_2$: C 74.65, H 6.48, N 12,01%; found: C 74.46, H 6.63, N 11.72%.

5,6-Dimethyl-9-methoxy-1-{4-[N-[3-(N,N-dimethylamino)propyl]carbamoyl]phenyl}-6H-pyrido[4,3-b]carbazole (**14**)

14: Yield 0.060 g (25%), m.p. 104–105°C. ^{1}H NMR (DMSO-d_6, δ, ppm): 1.70 (m, 2H, β-CH_2), 2.15 (s, 6H, -N(CH_3)$_2$), 2.30 (m, 2H, ă-CH_2), 3.10 (s, 3H, 5-CH_3), 3.45 (m, 2H, α-CH_2), 3.81 (s, 3H, 9-OCH_3), 4.14 (s, 3H, 6-CH_3), 7.16 (dd, $J_{8\text{-}10}$ = 2.4 Hz, $J_{8\text{-}7}$ = 8.8 Hz, 1H, 8-H), 7.53 (d, 1H, $J_{7\text{-}8}$ = 8.9 Hz, 7-H), 7.73 (d, $J_{10\text{-}8}$ = 2.4 Hz, 1H, 10-H), 7.79 (d, J = 8.1 Hz, 2H, 6'-H, 2'-H), 8.05 (m, 3H, 4-H, 5'-H, 3'-H), 8.48 (d, $J_{3\text{-}4}$ = 6.2 Hz, 1H, 3-H), 8.61 (s, 1H, 11-H), 8.67 (m, 1H, -CO**NH**CH_2). Analysis: calcd. for $C_{30}H_{32}N_4O_2$: C 74.97, H 6.71, N 11.66%; found: C 74.72, H 6.83, N 11.40%.

5,6-Dimethyl-9-methoxy-1-{3-[N-[2-(N,N-dimethylamino)ethyl]carbamoyl]phenyl}-6H-pyrido[4,3-b]carbazole (**15**)

15: Yield 0.062 g (27%), m.p. 210°C. ^{1}H NMR (DMSO-d_6, δ, ppm): 2.15 (s, 6H, -N(CH_3)$_2$), 2.40 (m, 2H, β-CH_2), 3.10 (s, 3H, 5-CH_3), 3.36 (m, 2H, α-CH_2), 3.80 (s, 3H, 9-OCH_3), 4.14 (s, 3H, 6-CH_3), 7.16 (dd, $J_{8\text{-}10}$ = 2.5 Hz, $J_{8\text{-}7}$ = 8.8 Hz, 1H, 8-H), 7.53 (d, $J_{7\text{-}8}$ = 8.8 Hz, 1H, 7-H), 7.67 (m, 2H, 10-H, 5'-H), 7.84 (m, 1H, 6'-H), 8.04 (m, 2H, 4-H, 4'-H), 8.19 (m, 1H, 2'-H), 8.48 (d, $J_{3\text{-}4}$ = 6.2 Hz, 1H, 3-H), 8.56 (m, 2H, -CO**NH**CH_2, 11-H). Analysis: calcd. for: $C_{29}H_{30}N_4O_2$: C 74.65, H 6.48, N 12.01; found: C 74,32, H 6.65, N, 12.24%.

5,6-Dimethyl-9-methoxy-1-{3-[N-[3-(N,N-dimethylamino)propyl]carbamoyl]phenyl}-6H-pyrido[4,3-b]carbazole (**16**)

16: Yield 0.081 g (34%), m.p. (dec.) 220°C. ^{1}H NMR (DMSO-d_6, δ, ppm): 1.76 (m, 2H, β-CH_2), 2.30 (s, 6H, -N(CH_3)$_2$), 2.55 (m, 2H, β-CH_2), 3.14 (s, 3H, 5-CH_3), 3.40 (m, 2H, α-CH_2), 3.79 (s, 3H, 9-OCH_3), 4.14 (s, 3H, 6-CH_3), 7.16 (dd, $J_{8\text{-}10}$ = 2.4 Hz, $J_{8\text{-}7}$ = 8.8 Hz, 1H, 8-H), 7.53 (d, $J_{7\text{-}8}$ = 8.8 Hz, 1H, 7-H), 7.66 (m, 2H, 10-H, 5'-H), 7.85 (m, 1H, 6'-H), 8.07 (m, 2H, 4-H, 4'-H), 8.25 (m, 1H, 2'-H), 8.47 (d, $J_{3\text{-}4}$ = 6.1 Hz, 1H, 3-H), 8.58 (s, 1H, 11-H), 8.77 (m, 1H, CO**NH**CH_2). Analysis: calcd. for: $C_{30}H_{32}N_4O_2$: C 74.97, H 6.71, N 11,66%; found: C 75,27, H 6.52, N 11.46%.

5,6-Dimethyl-9-methoxy-1-{3-[N-(2-hydroxy-1,1-dimethylethyl)carbamoyl]phenyl}-6H-pyrido[4,3-b]carbazole (**17**)

17: Yield 0.060 g (26%), m.p. 250–252°C. ^{1}H NMR (DMSO-d_6, δ, ppm) d: 1.31 (s, 6H, -(CH_3)$_2$) 3.10 (s, 3H, 5-CH_3), 3.51 (d, J = 5.9 Hz, 2H, -**CH_2**OH), 3.81 (s, 3H, 9-OCH_3), 4.14 (s, 3H, 6-CH_3), 4.90 (m, J = 5.9 Hz, 1H, OH), 7.16 (dd, $J_{8\text{-}10}$ = 2.4 Hz, $J_{8\text{-}7}$ = 8.8 Hz, 1H, 8-H), 7.53 (d, $J_{7\text{-}8}$ = 8.8 Hz, 1H, 7-H), 7.67 (m, 2H, 10-H, 5'-H), 7.82 (d, J = 7.6 Hz, 1H, 6'-H), 7.98 (d, J = 7.6 Hz 1H, 4'-H), 8.04 (d, $J_{4\text{-}3}$ = 6.2 Hz, 1H, 4-H), 8.14 (s, 1H, 2'-H), 8.48 (d, $J_{3\text{-}4}$ = 6.2 Hz, 1H, 3-H), 8.59 (s, 1H, 11H), 9.10 (s, 1H, CONH). Analysis: calcd. for: $C_{29}H_{29}N_3O_3$: C 74.50, H 6.25, N 8.99; found: C 74,30, H 6.15, N 9.44%.

5,6-Dimethyl-9-hydroxy-1-{4-[N-[3-(N,N-dimethylamino)propyl]carbamoyl]phenyl}-6H-pyrido[4,3-b]carbazole (**22**)

22: Yield 0.079 g (34%), m.p. 114–115°C. ^{1}H NMR (DMSO-d_6, δ, ppm): 1.72 (m, 2H, β-CH_2), 2.20 (s, 6H, -N(CH_3)$_2$), 2.35 (m, 2H, ă-CH_2), 3.10 (s, 3H, 5-CH_3), 3.23 (m, 2H, α-CH_2), 4.12 (s, 3H, 6-CH_3), 7.03 (dd, $J_{8\text{-}10}$ = 2.3 Hz, $J_{8\text{-}7}$ = 8.8 Hz, 1H, 8-H), 7.43 (m, 2H, 7-H, 10-H), 7.79 (d, J = 8.1 Hz, 2H, 5'-H, 3'-H), 8.04 (m, 3H, 4-H, 6'-H, 2'-H), 8.43 (s, 1H, 11-H), 8.48 (d, $J_{3\text{-}4}$ = 6.2 Hz, 1H, 3-H), 8.69 (m, 1H, -CO**NH**CH_2), 9.13 (s, 1H, 9-OH). Analysis: calcd. for: $C_{29}H_{30}N_4O_2$: C 74.65, H 6.48, N 12.01%; found: C 74,51, H 6.63, N 11.68%.

5,6-Dimethyl-9-hydroxy-1-{3-[N-[2-(N,N-dimethylamino)ethyl]carbamoyl]phenyl}-6H-pyrido[4,3-b]carbazole (**23**)

23: Yield 0.074 g (33%) of white hygroscopic crystals. ^{1}H NMR (DMSO-d_6, δ, ppm): 2.12 (s, 6H, -N(CH_3)$_2$), 2.26 (m, 2H, β-CH_2), 3.15 (m, 2H, α-CH_2), 3.42 (s, 3H, 5-CH_3), 4.13 (s, 3H, 6-CH_3), 7.03 (dd, $J_{8\text{-}10}$ = 2.5 Hz, $J_{8\text{-}7}$ = 8.8 Hz, 1H, 8-H), 7.39 (s, 1H, 10H), 7.44 (d, $J_{7\text{-}8}$ = 8.8 Hz, 1H, 7-H), 7.69 (d, J = 7.6 Hz 1H, 5'-H), 7.87 (m, 2H, 6'-H, 4'-H), 8.05 (d, $J_{4\text{-}3}$ = 6.2 Hz,1H, 4-H), 8.18 (s, 1H, 2'-H), 8.41 (s, 1H, 11-H), 8.48 (d, $J_{3\text{-}4}$ = 6.2 Hz, 1H, 3-H), 8.54 (m, 1H, CO**NH**CH_2,). Analysis: calcd. for:

$C_{28}H_{28}N_4O_2$: C 74.31, H 6.24, N, 12.38%; found: C 74,65, H 6.15, N 12.00%.

5,6-Dimethyl-9-hydroxy-1-{3-[N-(2-hydroxy-1,1-dimethylethyl)carbamoyl]phenyl}-6H-pyrido[4,3-b]carbazole (**24**)

24: Yield 0.061 g (27%), m.p. 259–260°C. ^{1}H NMR (DMSO-d_6, δ, ppm): 1.31 (s, 6H, -$(CH_3)_2$) 3.11 (s, 3H, 5-CH_3), 3.50 (d, J = 5.9 Hz, 2H, -**CH_2**OH), 4.14 (s, 3H, 6-CH_3), 4.85 (m, 1H, OH), 7.05 (dd, $J_{8\text{-}10}$ = 2.4 Hz, $J_{8\text{-}7}$ = 8.8 Hz, 1H, 8-H), 7.51 (d, $J_{7\text{-}8}$ = 8.8 Hz, 1H, 7-H), 7.68 (m, 1H 5'-H), 7.84 (m, 2H, 10-H, 6'-H), 8.09 (d, J = 7.6 Hz 1H, 4'-H), 8.09 (d, $J_{4\text{-}3}$ = 6.2 Hz 1H, 4-H), 8.13 (s, 1H, 2'-H), 8.48 (d, $J_{3\text{-}4}$ = 6.2 Hz, 1H, 3-H), 8.42 (s, 1H, 11H), 9.14 (s, 1H, -CONH). Analysis: calcd. for: $C_{28}H_{27}N_3O_3$: C 74.15, H 6.00, N 9.26%; found: C 73,96, H 6.15, N 9.73%.

5,6-Dimethyl-9-hydroxy-1-(4-carboxyphenyl)-6H-pyrido[4,3-b]carbazole (**18**), 5,6-dimethyl-9-hydroxy-1-(4-methoxycarbonylphenyl)-6H-pyrido[4,3-b]carbazole (**20**) and 5,6-dimethyl-9- hydroxy-1-(3-methoxycarbonylphenyl)-6H-pyrido[4,3-b]carbazole (**21**)

Compound **11** (0.20 g, 0.5 mmol) or **12** (0.20 g, 0.5 mmol) was dissolved in 100 mL of CH_2Cl_2, chilled to –70°C and boron tribromide (10 mL) was added dropwise at this temperature. The reaction mixture was stirred under nitrogen at normal pressure for 2 h, maintaining the temperature of –70°C. Then, the mixture was stirred at room temperature for 12 h and evaporated to dryness. The residue was taken up to water (50 mL), basified with $NaHCO_3$, extracted with CH_2Cl_2, and the extract was dried with magnesium sulfate. After evaporation of the solvent, the solid remaining was purified by chromatography on silica gel column and eluted with CH_2Cl_2 : CH_3OH (98 : 2, v/v) to give pure compound **20** or **21.**

Acetic acid was added to the water layer and extracted with 200 mL of CH_2Cl_2, and then dried over magnesium sulfate. After evaporation of the solvent, the solid was purified by chromatography on silica gel column and eluted with CH_2Cl_2 : CH_3OH (95 : 5, v/v) giving compound **18**

20: Yield 0.029 g (15%), m.p. 243°C. ^{1}H NMR (DMSO-d_6, δ, ppm): 3.08 (s, 3H, 5-CH_3), 3.93 (s, 3H, -COO**CH_3**), 4.11 (s, 3H, 6-CH_3), 7.03 (dd, $J_{8\text{-}10}$ = 2.3 Hz, $J_{8\text{-}7}$ = 8.7 Hz, 1H, 8-H), 7.42 (d, $J_{7\text{-}8}$ = 8.7 Hz, 1H, 7-H), 7.45 (d, $J_{10\text{-}8}$ = 2.4 Hz, 1H, 10-H), 7.86 (d, J = 8.3 Hz, 2H, 5'-H, 3'-H), 8.05 (d, $J_{4\text{-}3}$ = 6.2 Hz, 1H, 4-H), 8.17 (d, J = 8.3 Hz, 2H, 6'-H, 2'-H), 8.43 (s, 1H, 11-H), 8.48 (d, $J_{3\text{-}4}$ = 6.2 Hz, 1H, 3-H), 9.09 (s, 1H, 9-OH). Analysis: calcd. for: $C_{25}H_{20}N_2O_3$: C 75.74, H 5.08, N 7.07%; found: C 75.44, H 5.34, N 7.65%.

21: Yield 0.082 g (41.5%), m.p. (dec.) 235°C. ^{1}H NMR (DMSO-d_6, δ, ppm): 3.09 (s, 3H, 5-CH_3), 3.89 (s, 3H, -COO**CH_3**), 4.11 (s, 3H, 6-CH_3), 7.03 (dd, $J_{8\text{-}10}$ = 2.2 Hz, $J_{8\text{-}7}$ = 8.6 Hz, 1H, 8-H), 7.43 (m, 2H, 7-H, 10-H), 7.76 (m, J = 7.7 Hz, 1H, 5'-H), 8.03 (m, 2H, 4-H, 6'-H), 8.15 (d, J = 7.7 Hz, 1H, 4'-H), 8.27 (s, 1H, 2'H), 8.43 (s, 1H, 11-H), 8.48 (d, $J_{3\text{-}4}$ = 6.2 Hz, 1H, 3-H), 9.12 (s, 1H, 9-OH). Analysis: calcd. for: $C_{25}H_{20}N_2O_3$: C 75.74, H 5.08, N 7.07%; found: C 76.03, H 4.88, N 6.63%.

18: Yield: 0.038 g (20%), m.p. (dec.) 262°C. ^{1}H NMR (DMSO-d_6, δ, ppm): 3.08 (s, 3H, 5-CH_3), 4.11 (s, 3H, 6-CH_3), 7.03 (d, $J_{8\text{-}7}$ = 8.7 Hz, 1H, 8-H), 7.43 (d, $J_{7\text{-}8}$ = 8.8 Hz, 1H, 7-H), 7.45 (s, 1H, 10-H), 7.82 (d, J = 8.3 Hz, 2H, 5'-H, 3'-H), 8.04 (d, $J_{4\text{-}3}$ = 6.2 Hz, 1H, 4-H), 8.16 (d, J = 8.2 Hz, 2H, 6'-H, 2'-H), 8.43 (s, 1H, 11-H), 8.48 (d, $J_{3\text{-}4}$ = 6.2 Hz, 1H, 3-H), 9.12 (s, 1H, 9-OH). Analysis: calcd. for: $C_{24}H_{18}N_2O_3$: C 75.38, H 4.74, N 7.33%; found: C 75.13, H 5.47, N 7.11%.

5,6-Dimethyl-9-hydroxy-1-{4-[N-[2-(N,N-dimethylamino)ethyl]carbamoyl]phenyl}-6H-pyrido[4,3-b]carbazole (**19**)

A mixture of compound **18** 0.19 g (0.5 mmol), THF (20 mL) and 0.89 g (0.55 mmol) of N,N'-carbonyldiimidazole was stirred at room temperature for 0.5 h. Then N,N-dimethylethylenediamine (10 mL) and a few drops of DMF were added and the resulting mixture was refluxed for 0.5 h. Then the reaction mixture was stirred for 24 h and after evaporation to dryness, the residue was purified by column chromatography on silica gel column with CH_2Cl_2 : CH_3OH (95 : 5, v/v) as eluent.

19: Yield 0.058 g (26%), m.p. 160°C. ^{1}H NMR (DMSO-d_6, δ, ppm): 2.20 (s, 6H, -$N(CH_3)_2$), 2.55 (m, 2H, β-CH_2), 3.08 (s, 3H, 5-CH_3), 3.42 (m, 2H, α-CH_2), 4.11 (s, 3H, 6-CH_3), 7.03 (dd, $J_{8\text{-}10}$ = 2.4 Hz, $J_{8\text{-}7}$ = 8.7 Hz, 1H, 8-H), 7.42 (m, 2H, 7-H, 10-H), 7.79 (m, J = 8.2 Hz, 2H, 5'-H, 3'-H), 8.04 (m, 3H, 4-H, 6'-H, 2'-H), 8.42 (s, 1H, 11-H), 8.48 (d, $J_{3\text{-}4}$ = 6.2 Hz, 1H, 3-H), 8.57 (m, 1H, -CO**NH**CH_2). Analysis: calcd. for: $C_{28}H_{28}N_4O_2$: C 74.31, H 6.24, N 12,38%; found: C 74.61, H 6.06, N, 12.48%.

Biological tests

Test solutions of 11 new pyrido[4,3-*b*]carbazole derivatives (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.

Table 1. Cell growth inhibition on L1210, A498, A549, HT29. IC_{50} values [μM] ± SD of compounds **13-24** compared with ellipticine.

Compound no.	**ID_{50} [μM] ± SD**			
	L1210	**A498**	**A549**	**HT29**
13	6.12 ± 0.47	6.62 ± 0.57	5.35 ± 1.88	5.33 ± 1.77
14	5.70 ± 0.33	6.90 ± 0.39	4.30 ± 1.95	4.93 ± 1.64
15	6.17 ± 0.83	4.77 ± 2.87	2.70 ± 1.60	3.94 ± 1.20
16	38.15 ± 26.7	-	-	-
17	6.11 ± 1.28	5.62 ± 1.41	2.41 ± 0.53	3.03 ± 0.89
19	7.15 ± 2.76	6.45 ± 1.10	8.19 ± 1.41	6.78 ± 1.74
20	inactive	-	-	-
21	72.5 ± 44.9	-	-	-
22	6.76 ± 1.39	5.17 ± 0.35	7.38 ± 2.07	9.30 ± 2.67
23	6.08 ± 3.96	6.83 ± 0.53	8.25 ± 2.14	8.55 ± 1.71
24	5.82 ± 2.75	6.41 ± 1.25	2.33 ± 1.52	7.16 ± 3.13
Ellipticine	2.43 ± 0.73	1.74 ± 0.04	0.85 ± 0.04	1.66 ± 0.04

Cell lines

The L1210 (murine leukemia), A498 (kidney cancer), 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, A498 and HT-29 cells 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 A498 and 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) or SRB (for A549, A498 and HT29) methods were used as described by Skehan et al. (20). The cytostatic assays were performed after 96-h 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

Chemical synthesis of the newly obtained compounds was usually performed using described previously procedures (21) except of the synthesis of compounds **11**, **12** and **19**. Derivatives **11** and **12** can be obtained from **9** and **10** using dimethyl carbonate as methylating agent and Adogen 464 as a transfer phase catalyst. Application of this catalyst seems to be more effective than used previously by us 18-crown-6, resulting in remarkably shorter reaction time and slightly better yield of products. Compound **19** was synthesized successfully directly from the corresponding carboxylic acid **18** using method adapted from standard peptide synthesis with N,N'-carbonyldiimidazole as a coupling agent. The structures of 11 biologically tested compounds are shown in Figure 3. Their cytostatic activities expressed as a half maximal inhibitory concentration are summarized in Table 1 and compared with reference drug ellipticine. Eight of them exhibited remarkable but not strong activity, usually about 5 times weaker than the reference. Considering standard deviation values there are no significant differences in activity between compounds **13**, **14**, **15**, **17**, **19**, **22**, **23** and **24** except of compounds **15** and **17,** which were approximately twice as active as the others against A549 and HT29 tumor cell lines. On the other hand, three of our derivatives namely starting methyl esters **20** and **21** and compound **16** which is 3' substituted carbamoyl derivative, showed very weak or no activity.

These data suggest that the carbamoyl moieties at 3' and 4' positions of 1-phenyl-6H-pyrido[4,3-*b*]carbazole ring system can enable cytostatic activity of the synthesized compounds in comparison with respective methoxycarbonyl derivatives **20** and **21**. Cytostatic properties of the active amides are very similar to each other and only slightly depend on their substitution position or chain length of the substituents. As it was previously described in our earlier work (21), the more advantageous structure modification in this class of compounds are small electron donor substituents (OH or NH_2) at 3' or 4' position or nitro group at 4' position (19).

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