DOI 10.1002/ardp.200500141 Arch. Pharm. Chem. Life Sci. 2005, 338, 556–561

Synthesis of 5,6-Dimethyl-9-methoxy-1-phenyl-6*H*-pyrido[4,3-*b*]carbazole Derivatives and their Cytotoxic Activity

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Starting from 2-(6-methoxy-1-methyl-9*H*-carbazol-2-yl)ethylamine **7** and mixed anhydrides of 4-nitrobenzoic acid or 4-methoxybenzoic acid, the corresponding 5,6-dimethyl-9-methoxy-1-(4-substituted phenyl)-6*H*-pyrido[4,3-*b*]carbazoles **11a−b**, 5,6-dimethyl-9-hydroxy-1-(4-substituted phenyl)-6*H*-pyrido[4,3-*b*]carbazoles **12a, 12c,** and their quaternary salts **13a−d** were obtained. The four new pyridocarbazole derivatives **12a−c** and **13d** satisfy the international activity criterion for synthetic compounds, namely an ID_{50} value lower then 4 μg/mL in preliminary *in vitro* cytotoxic activity screening against the A549 cell line (non-small cell lung cancer).

Keywords: Olivacine; 1-Substituted 6*H*-pyrido[4,3-*b*]carbazole; Cytotoxicity

Received: May 25, 2005; Accepted: July 29, 2005

Introduction

It has been known for almost forty years that the natural alkaloids Ellipticine **1** and Olivacine **2** (Figure 1) exert anti-neoplastic activity. Structure modifications of the above-mentioned alkaloids have since been conducted in the search for new derivatives with advantageous antitumor activity in the pyrido[4,3-*b*]carbazole ring system. Numerous derivatives of pyrido[4,3-*b*] carbazole have shown strong anticancer activity in *in vitro* and *in vivo* studies [1, 2]. So far, only the acetate of 9-hydroxy-2-methylellipticinium **3** (Figure 2) has found application in clinical treatment [3].

Much less attention has been paid to the synthesis of analogues of the alkaloid Olivacine **2,** which has also been proved to possess potential anticancer properties. One of

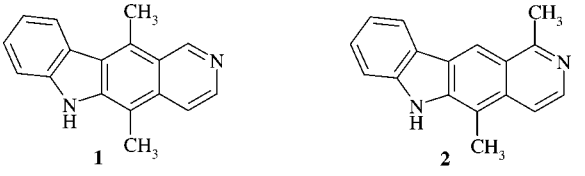

Figure 1. Structure of natural alkaloids Ellipticine **1** and Olivacine **2**.

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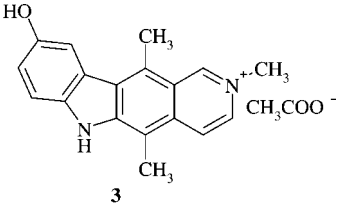


Figure 2. Structure of acetate of 9-hydroxy-2-methylellipticinium **3**.

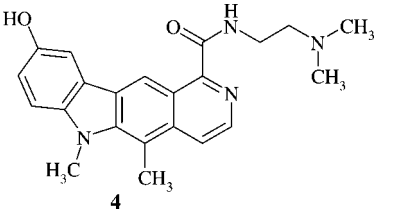


Figure 3. Analogue of alkaloid Olivacine **2**.

these (**4**, Figure 3) is in the course of clinical trials [4, 5]. 1-Pyridylsubstituted pyrido[4,3-*b*]carbazole derivatives **5** (Figure 4) have shown strong cytostatic activity [6−8].

According to existing results, the sole attempt to synthesize 1-(4-chlorophenyl)-substituted derivative of the above mentioned ring system, resulted in 1-phenyl-5-methyl-6*H*-pyrido[4,3-*b*] carbazole, which was inactive against the

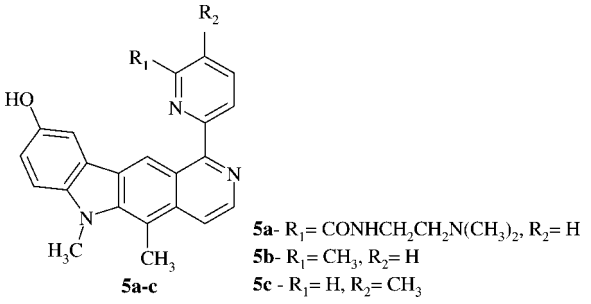


Figure 4. Structure of 1-pyridylsubstituted pyrido[4,3-*b*]carbazole derivative **5**.

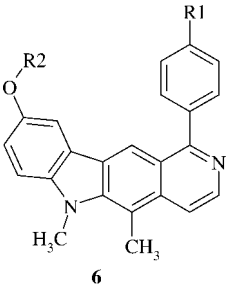
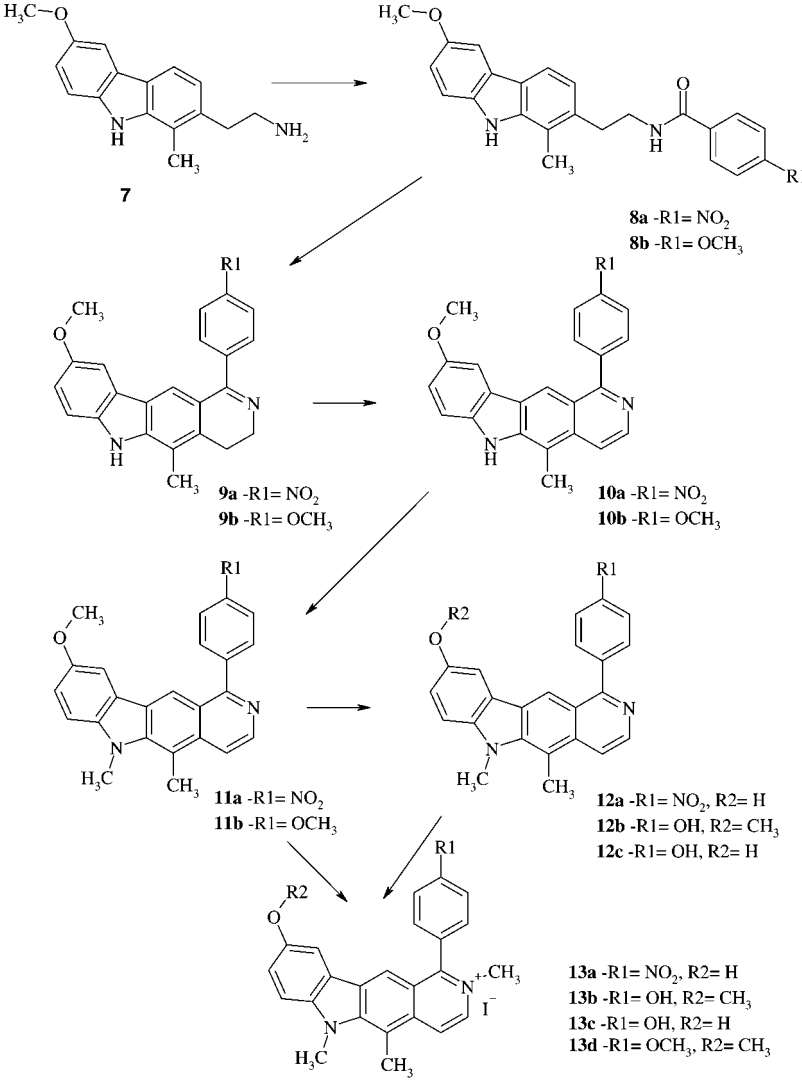


Figure 5. Structure of 1-phenyl-substituted derivative of type **6**.

L1210 mouse leukemia cell line [9]. Because the above-mentioned 1-pyridylsubstituted pyridocarbazoles, having substituents in the aromatic pyridyl group, exerted significant activity in identical research, it was decided to synthesize new 1-phenyl-substituted derivatives of type **6** (Figure 5) and verify their cytotoxic activity. These pyrido[4,3-*b*]-carbazole derivatives **6** were obtained according to Scheme 1.



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

Full Paper

Results and discussion

Synthetic chemistry

The starting compound, 2-(6-methoxy-1-methyl-9*H*-carbazol-2-yl)ethylamine **7**, has already been described [4]. It was allowed to react with mixed anhydride of 4-nitrobenzoic acid. Cyclization of the resulting amide **8a** with phosphorous oxychloride in boiling toluene gave 9-methoxy-5-methyl-1-(4-nitrophenyl)-3,4-dihydro-6*H*-pyrido[4,3-*b*]carbazole **9a**, which was aromatized to derivative **10a** by dehydrogenation over 10% palladium on charcoal in boiling diphenyl ether. *N*-6-Methylation of **10a** to **11a** was performed using an excess of dimethyl carbonate in dimethylformamide, in the presence of potassium carbonate and 18-Crown-6. The compound **11a** was 9-*O*-demethylated to 5,6-dimethyl-9-hydroxy-1-(4-nitrophenyl)-6*H*-pyrido[4,3-*b*]-carbazole **12a** by heating with an 48% aqueous solution of hydrobromic acid.

An analogous sequence of reactions was carried out using mixed anhydride of 4-methoxybenzoic acid as the acylating agent for amine **7** and adequate amide **8b** was obtained. Compound **8b** was boiled in toluene with $POCl_3$ and the cyclization product a 3,4-dihydroderivative of the pyrido[4,3-*b*]carbazole ring system **9b** was obtained, which was aromatized to derivative **10b** and then *N*-6 methylated to 9-methoxy-1-(4-methoxyphenyl)-5,6-dimethyl-6*H*-pyrido[4,3-*b*]carbazole **11b**. The same protocol for the demethylation of **11a** was used and led to a mixture of the 1-(4-hydroxyphenyl)-9-methoxy derivative **12b** and the 1-(4-hydroxyphenyl)-9-hydroxy derivative **12c**. Quaternization of the pyridinic nitrogens of compounds **11b** and **12a**–**c** into the corresponding salts **13a**–**d** was achieved with methyl iodide in DMF [10].

Biology

The nine new 1-substituted-6*H*-pyrido[4,3-*b*]carbazole derivatives **11a**–**13d** were subjected to preliminary *in vitro* cytotoxic activity screening against the A549 cell line (non-small cell lung cancer). All the new tested compounds exhibited different biological properties, depending on the kind of substituent at positions 1 and 9 in the main heterocyclic system.

Based on the results assembled in Table 1, it can be seen that replacement of the studied ring system of the phenyl group at the 1-position by *p*-hydroxy, *p*-methoxy, or *p*-nitrophenyl groups has caused reactivation of the stripped activity of 1-phenylsubstituted pyrido[4,3-*b*] carbazole. The boost in the hydrophilic properties of the studied compounds (**11b**, **12a**–**c**) by their transformation on quaternary salts (**13a**–**d**) did not cause the expected increase in activity. A decline in the cytotoxic activity of the obtained compounds in the studied range of concentrations generally resulted. It was possible to see a tripling in growth activity only in the case of compound **13d** compared with the starting derivative **11b**. Compound **13c** was inactive in this concentration range.

Table 1. Cell growth inhibition of A549 cells (non-small cell lung cancer), ID_{50} values (μg/mL) of 1-substituted-6H-pyrido[4,3-b]carbazole **11a**–**13d** compared to **5c**.

Compound No	ID_{50} (μg/mL)	IC_{50} (μM)
5c	3.38 ± 0.54	9.56 ± 1.52
11a	3.78 ± 2.06	9.51 ± 5.18
11b	4.925 ± 2.22	12.87 ± 5.80
12a	0.84 ± 1.21	2.37 ± 3.41
12b	2.305 ± 0.43	6.25 ± 1.16
12c	0.625 ± 0.02	1.76 ± 0.056
13a	10.25 ± 1.04	20.69 ± 2.099
13b	3.05 ± 1.15	5.97 ± 2.25
13c	inactive	
13d	2.51 ± 0.93	4.78 ± 1.77

Conclusion

The results of the *in vitro* study may be summarized as follows:

The compounds **11a**–**b** and **12b,** containing a methoxy group at position 9 in the pyrido[4,3-*b*] carbazole ring system, exhibit lower cytotoxic activity than their 9-hydroxy equivalents **12a** and **12c.**

The compounds **12a**–**c**, **13b,** and **13d** were more active than the reference derivative **5c**.

The four new pyridocarbazole derivatives **12a**–**c** and **13d** satisfy the international activity criterion for synthetic compounds, namely an ID_{50} value lower then 4 μg/mL [11].

Acknowledgments

The technical assistance of B. Biaduń is acknowledged. The presented study was supported by the Wrocław University of Medicine (internal project number 434).

Experimental

Chemistry

Melting points were determined on a Köfler apparatus (C. Reichert, Vienna, Austria) and are uncorrected; ^1H NMR spectra were recorded on a Tesla BS 587 A at 80 MHz or on a Bruker 300 at 300 MHz (Bruker, Rheinstetten, Germany). 14 MHz, using TMS as the 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. The starting 2-(6-methoxy-1-methylcarbazol-2-yl)ethylamine **7** (Scheme 1) was prepared according to a described procedure [4].

*General procedure for the synthesis of N-[2-(6-methoxy-1-methyl-9H-carbazol-2-yl)ethyl]-4-substituted benzamides **8***

Triethylamine (0.506 g, 5 mmol) was added to the appropriate 4-nitro-or 4-methoxybenzoic acid (4.4 mmol) in dry THF (100 mL). After cooling to −10 °C, a solution of ethyl chloroformiate (0.543 g, 5 mmol) in dry THF (10 mL) was added to the resulting mixture under stirring. The mixture was stirred for a further 30 min and then a solution of 2-(6-methoxy-1-methylcarbazol-2-yl)ethylamine **7** (1.016 g, 4 mmol) in THF (100 mL) was added drop-wise at −10 °C. The resulting mixture was left to reach room temperature (20 h) under stirring and was then evaporated to dryness. The residue was taken up in water (50 mL), basified with concentrated aqueous ammonia, extracted with methylene dichloride, and then dried over magnesium sulfate. Evaporation of the solvent provided a solid residue, which was recrystallized in the given solvent.

8a: Yield 52.9%, mp 223−224 °C (from ethanol). Anal. calcd. for $C_{24}H_{24}N_3O_4$: C, 68.47; H, 5.25; N, 10.42. Found: C, 68.60; H, 5.40; N, 10.13. ^{1}H NMR (DMSO-d_6) δ: 2.53 (s, 3H, 1-CH_3), 3.03 (m, 2H, α-CH_2), 3.50 (m, 2H, β-CH_2), 3.83 (s, 3H, 6-OCH_3), 6.98 (m, 2H, 3-H + 7-H), 7.37 (d, $J_{8\text{-}7}$ = 8.8 Hz, 1H, 8-H), 7.58 (d, $J_{5\text{-}7}$ = 2.4 Hz, 1H, 5-H), 7.83 (d, $J_{4\text{-}3}$ = 7.8 Hz, 1H, 4-H), 8.07 (d, J = 8.8 Hz, 2H, Phenyl-H), 8.32 (d, J= 8.8 Hz, 2H, Phenyl-H), 8.97 (t, 1H, β- NH), 10.82 (s, 1H, 9-H).

8b: Yield 86.4%, mp 243−244 °C (from ethanol). Anal. calcd. for $C_{24}H_{24}N_2O_3$: C, 74.21; H, 6.23; N, 7.21. Found: C, 73.96; H, 6.38; N, 6.97. ^{1}H NMR (DMSO-d_6) δ: 2.52 (s, 3H, 1-CH_3), 2.99 (m, 2H, α-CH_2), 3.45 (m, 2H, β-CH_2), 3.80 (s, 3H, 4′-OCH_3), 3.83 (s, 1H, 6-OCH_3), 6.98 (m, 4H, 3H + 7-H + Phenyl-H), 7.37 (d, $J_{8\text{-}7}$ = 8.8 Hz, 1H, 8-H), 7.58 (d, $J_{5\text{-}7}$ = 2.2 Hz, 1H, 5-H), 7.83 (m, 3H, 4-H + Phenyl-H), 8.47 (t, 1H, β-NH), 10.80 (s, 1H, 9-H).

*General procedure for the synthesis of 9-methoxy-5-methyl-1-(4-substituted phenyl)-3,4-dihydro-6H-pyrido[4,3-b]carbazoles **9***

Preceding amide **8a−b** (3 mmol) was dissolved in boiling toluene (150 mL) and treated drop-wise with phosphorous oxychloride (12 mL). Reflux was continued for a 12-h period, and evaporation under reduced pressure afforded a residue, which was taken up in water (100 mL), basified to pH 9-10 with concentrated aqueous ammonia, and extracted with methylene dichloride. Evaporation of the solvent provided a solid residue, which was recrystallized in the given solvent.

9a: Yield 94.2%, mp 279 °C (from ethanol). Anal. calcd. for $C_{23}H_{19}N_3O_3$: C, 71.68; H, 4.97; N, 10.90. Found: C, 71.53; H, 5.12; N, 10.57. ^{1}H NMR (DMSO-d_6) δ: 2.53 (s, 3H, 5-CH_3), 2.87 (t, $J_{4\text{-}3}$ = 6.4Hz, 2H, 4-H), 3.76 − 3.79 (m, 5H, 9-OCH_3 + 3-H), 6.99 (dd, $J_{8\text{-}7}$ = 8.6 Hz, $J_{8\text{-}10}$ = 1.9 Hz, 1H, 8-H), 7.41 (d, $J_{7\text{-}8}$ = 8.5 Hz, 1H, 7-H), 7.57 (d, $J_{10\text{-}8}$ =1.8 Hz, 1H, 10-H), 7.76 (s, 1H, 11-H), 7.85 (d, J = 8.8 Hz, 2H, Phenyl-H), 8.34 (d, J = 8.8 Hz, 2H, Phenyl-H) 11.27 (s, 1H, 6-H).

9b: Yield 89.8%, mp 261-262 °C (from ethanol). Anal. calcd. for $C_{24}H_{22}N_2O_2$: C, 77.81; H, 5.99; N, 7.56. Found: C, 77.53; H, 6.07; N, 7.35. ^{1}H NMR (DMSO-d_6) δ: 2.52 (s, 3H, 5-CH_3), 2.88 (m, 2H, 4-H), 3.69 (m, 2H, 3-H), 3.78 (s, 3H, 4′-OCH_3), 3.85 (s, 1H, 9-OCH_3), 7.00 (dd, $J_{8\text{-}7}$ = 8.6 Hz, $J_{8\text{-}10}$ = 2.4 Hz, 1H, 8-H), 7.07 (d, J = 8.6 Hz, 2H, Phenyl-H), 7.42 (d, 1H, 7-H), 7.58 (m, 3H, 10-H , Phenyl-H), 7.89 (s, 1H, 11-H), 11,35 (s, 1H, 6-H).

*General procedure for the synthesis of 9-methoxy-5-methyl-1-(4-substituted phenyl)-6H-pyrido[4,3-b] carbazoles **10***

The required compound **9a-b** (3 mmol) was refluxed in diphenyl ether (50 mL) in the presence of 10% palladized charcoal (0.1 g) for 45 min. The catalyst was filtered off and the filtrate was cooled and diluted with hexane. The resulting precipitate was collected and washed with hexane and recrystallized in the given solvent.

10a: Yield 51.6%, mp 298 °C. Anal. calcd. for $C_{23}H_{17}N_3O_3$: C, 72.05; H, 4.47; N, 10.96. Found: C, 71.87; H, 4.66; N, 10.73. ^{1}H NMR (DMSO-d_6) δ: 2.87 (s, 3H, 5-CH_3), 3.82 (s, 3H, 9-OCH_3), 7.14 (dd, $J_{8\text{-}7}$ = 8.8 Hz, $J_{8\text{-}10}$ = 2.4 Hz, 1H, 8-H), 7.45 (d, $J_{7\text{-}8}$ = 8.8 Hz, 1H, 7-H), 7.84 (d, $J_{10\text{-}8}$ = 2.4 Hz, 1H, 10-H), 8.00−8.05 (m, 3H, 4-H + Phenyl-H), 8,46 (d, J = 8.8 Hz, 2H, Phenyl-H), 8.51 (d, $J_{3\text{-}4}$ = 6.3 Hz, 1H, 3-H), 8.61 (s, 1H, 11-H), 11.30 (s, 1H, 6-H).

10b: Yield 80.4%, mp 250-251 °C (ethyl acetate). Anal. calcd. for $C_{24}H_{20}N_2O_2$: C, 78.24; H, 5.47; N, 7.60. Found: C, 78.03; H, 5.66; N, 7.51. ^{1}H NMR (DMSO-d_6) δ: 2.85 (s, 3H, 5-CH_3), 3.84 (s, 3H, 4′-OCH_3), 3.90 (s, 3H, 9-OCH_3), 7.13 (dd, $J_{8\text{-}7}$ = 8.8 Hz, $J_{8\text{-}10}$ = 2.4 Hz, 1H, 8-H), 7.17 (d, J = 8.8 Hz, 2H, Phenyl-H), 7.44 (d, $J_{7\text{-}8}$ =8.6 Hz, 1H, 7-H), 7.69 (d, J = 8.6 Hz, 2H, Phenyl-H), 7.74 (d, $J_{10\text{-}8}$ = 2.4 Hz, 1H, 10-H), 7.90 (d, $J_{4\text{-}3}$ = 6.1 Hz, 1H, 4-H), 8.43 (d, 1H, 3-H), 8.68 (s, 1H, 11-H), 11.20 (s, 1H, 6-H).

*General procedure for the synthesis of 5,6-dimethyl-9-methoxy-1-(4-substituted phenyl)-6H-pyrido[4,3-b]carbazoles **11***

A mixture of the required compound **10a−b** (2 mmol), finely powdered dry potassium carbonate (500 mg), dimethyl carbonate (15 mL), dimethylformamide (2 mL), and 18-crown-6-ether (3 drops) was heated at reflux under stirring for a 12-h period. After evaporation to dryness, the residue was taken up in water. The solid was collected, air-dried, and recrystallized in the given solvent.

11a: Yield 91.1%, mp 304 °C (ethyl acetate). Anal. calcd. for $C_{24}H_{19}N_3O_3$: C, 72.53; H, 4.82; N, 10.57. Found: C, 78.19; H, 5.69; N, 11.57. ^{1}H NMR (DMSO-d_6) δ: 3.15 (s, 3H, 5-CH_3), 3.83 (s, 3H, 9-OCH_3), 4.19 (s, 3H, 6-CH_3), 7.21 (dd, $J_{8\text{-}7}$ = 8.8 Hz, $J_{8\text{-}10}$ = 2.4 Hz, 1H, 8-H), 7.57 (d, 1H, 7-H), 7.85 (d, $J_{10\text{-}8}$ = 2.4 Hz, 1H, 10-H), 8.02 (d, J = 8.8 Hz, 2H, Phenyl-H), 8.14 (d, J_4-3 = 5.8 Hz, 1H, 4-H), 8.47 (d, J = 8.8 Hz, 2H, Phenyl-H), 8.54 (d, 1H, 3-H), 8.64 (s, 1H, 11-H).

11b: Yield 90.3%, mp 210−211 °C (from cyclohexane). Anal. calcd. for $C_{25}H_{22}N_2O_2$: C, 78.51; H, 5.80; N, 7.32. Found: C, 78.19; H, 5.95; N, 7.13. ^{1}H NMR (DMSO-d_6) δ: 3,10 (s, 3H, 5-CH_3), 3.84 (s, 3H, 4′-OCH_3), 3.89 (s, 3H, 9-OCH_3), 4.15 (s, 3H, 6-CH_3), 7.17 (m, 3H, 8-H + Phenyl-H), 7.54 (d, $J_{7\text{-}8}$ = 8.8 Hz, 1H, 7-H), 7.70 (d, J = 8.6 Hz, 2H, Phenyl-H), 7.73 (d, $J_{10\text{-}8}$ = 2.4 Hz, 1H, 10-H), 7.98 (d, $J_{4\text{-}3}$ = 6.4 Hz, 1H, 4-H), 8.45 (d, 1H, 3-H), 8.68 (s, 1H, 11-H).

*5,6-Dimethyl-9-hydroxy-1-(4-nitrophenyl)-6H-pyrido[4,3-b]carbazole **12a***

A mixture of the 9-methoxy derivative **11a** (1 mmol) and hydrobromic acid 48% (15 mL) was heated at reflux under stirring for 2 h. After evaporation to dryness, the residue was taken up in water. The resulting mixture was basified with concentrated ammonia, extracted with methylene dichloride, and then dried over magnesium sulfate. After evaporation of the solvent, the solid was recrystallized in the given solvent.

12a: Yield 71.2%; mp >300 °C (from methylene chloride). Anal. calcd. for $C_{23}H_{17}N_3O_3$: C, 72.05; H, 4.47; N, 10.96. Found: C, 71.93; H, 4.67; N, 10.75. ^{1}H NMR (DMSO-d_6) δ: 3.11 (s, 3H, 5-CH_3), 4.13 (s, 3H, 6-CH_3), 7.05 (dd, $J_{8\text{-}7}$ = 8.5 Hz, $J_{8\text{-}10}$ = 2.2 Hz, 1H, 8-H), 7.45 (d, 1H, 7-H), 7.53 (d, $J_{10\text{-}8}$ = 2.2 Hz, 1H, 10-H), 8.02 (d,

J = 8.8 Hz, 2H, Phenyl-H), 8.12 (d, J_{4-3} = 6.0 Hz, 1H, 4-H), 8.46 (m, 3H, Phenyl-H + 11-H), 8.53 (d, 1H, 3-H), 9.13 (s, 1H, 9-OH).

*9-Methoxy-1-(4-hydroxyphenyl)-5,6-dimethyl-6H-pyrido[4,3-b]-carbazole (***12b**) and 9-methoxy-1-(4-hydroxyphenyl)-5,6-dimethyl-6H-pyrido[4,3-b]carbazole ***12c***

A mixture of the 9-methoxy derivative **11b** (1 mmol) and hydrobromic acid 48% (15 mL) was heated at reflux under stirring for 2 h. After evaporation to dryness, the residue was taken up in water. The resulting mixture was basified with concentrated ammonia, extracted with methylene dichloride, and then dried over magnesium sulfate. After evaporation of the solvent, the residue was purified by column chromatography over a silica gel column. Elution with a methylene chloride/methanol 98:2 v/v mixture gave two products: a more mobile one, which corresponding to the monohydroxy derivative **12b** and a less mobile corresponding to the dihydroxy derivative **12c**.

12b: Yield 9.2%, mp 183-185°C (from isopropyl acetate). Anal. calcd. for $C_{24}H_{20}N_2O_2$: C, 78.24; H, 5.47; N, 7.60. Found: C, 78.03; H, 5.63; N, 7.47.^{1}H NMR (DMSO-d_6) δ: 3.09 (s, 3H, 5-CH_3), 3.89 (s, 3H, 9-OCH_3), 4.12 (s, 3H, 6-CH_3), 7.03 (dd, J_{8-7} = 8.8 Hz, J_{8-10} = 2.2 Hz, 1H, 8-H), 7.17 (d, J = 8.2 Hz, 2H, Phenyl-H), 7.43 (m, 2H, 7H + 10H), 7.69 (d, J = 7.9 Hz, 2H, Phenyl-H), 7.98 (d, J_{4-3} = 6.3 Hz, 1H, 4-H), 8.43 (d, 1H, 3-H), 8.52 (s, 1H, 11-H), 9.14 (s, 1H, 4′-OH).

12c: Yield 26.4%; mp >300°C (from isopropyl acetate). Anal. calcd. for $C_{23}H_{18}N_2O_2$: C, 77.95; H, 5.12; N, 7.90. Found: C, 77.73; H, 5.39; N, 7.73. ^{1}H NMR (DMSO-d_6) δ: 3.08 (s, 3H, 5-CH_3), 4.12 (s, 3H, 6-CH_3), 6.98 (d, J = 8.3 Hz, 2H, Phenyl-H), 7.05 (dd, J_{8-7} = 8.6 Hz, J_{8-10} = 2.2 Hz, 1H, 8-H), 7.44 (m, 1H, 7-H + 10H), 7.57 (d, J = 8.2 Hz, 2H, Phenyl-H), 7.96 (d, J_{4-3} = 6.1 Hz, 1H, 4-H), 8.42 (d, J_{3-4}= 6.1 Hz, 1H, 3-H), 8.54 (s, 1H, 11-H), 9.10 (s, 1H, 4′-OH), 9.77 (s, 1H, 9-OH).

General procedure for quaternization

The corresponding 1-(4-substituted phenyl)-6H-pyrido[4,3-b]carbazole (**11b**, **12a**−**12c**) (0.1 mmol) was dissolved in 3 mL of DMF (anhydrous) at room temperature and 0.06 mL (1 mmol) of iodomethane was added. The mixture was stirred for 2 h at room temperature, usually with the formation of an orange precipitate, and concentrated *in vacuo*. The precipitate was recrystallized from ethanol.

13a: Yield 96%; mp >300°C (from ethanol). Anal. calcd. for $C_{24}H_{20}N_3O_3I$: C, 54.87; H, 3.84; N, 8.00. Found: C, 54.63; H, 3.97; N, 7.73. ^{1}H NMR (DMSO-d_6) δ: 3.22 (s, 3H, 5-CH_3), 4.02 (s, 3H, 6-CH_3), 4.25 (s, 3H, 2-CH_3), 7.15 (dd, J_{8-7} = 8.9 Hz, J_{8-10} = 2.5 Hz, 1H, 8-H), 7.59 (d, 1H, 7-H), 7.66 (d, J_{10-8} = 2.5 Hz, 1H, 10-H), 8.05 (d, J = 8.5 Hz, 2H, Phenyl-H), 8.19 (s, 1H, 11-H), 8.61−8.66 (m, 3H, Phenyl-H + 4-H), 8.73 (d, J_3-4 = 7.4 Hz, 1H, 3-H), 9.33 (s, 1H, 9-OH).

13b: Yield 49%; mp >300°C (from ethanol). Anal. calcd. for $C_{25}H_{23}N_2O_2I$: C, 58.83; H, 4.54; N, 5.49. Found: C, 58.68; H, 4.73; N, 5.27. ^{1}H NMR (DMSO-d_6) δ: 3.19 (s, 3H, 5-CH_3), 3.95 (s, 3H, 9-OCH_3), 4.03 (s, 3H, 6-CH_3), 4.24 (s, 3H, 2-CH_3), 7.13 (dd, J_{8-7} = 8.7 Hz, 1H, 8-H), 7.33 (d, J = 8.7 Hz, 2H, Phenyl-H), 7.49 (d, J_{10-8} = 2.3 Hz, 1H, 10-H), 7.59 (d, J_{7-8} = 8.9 Hz, 1H, 7-H), 7.66 (d, J = 8.7 Hz, 2H, Phenyl-H), 8.16 (s, 1H, 11-H),), 8.63 (m, 2H, 3-H+ 4-H), 9.35 (s, 1H, 4′-OH).

13c: Yield 43%; mp >300°C (from ethanol). Anal. calcd. for $C_{24}H_{21}N_2O_2I$: C, 58.08; H, 4.26; N, 5.64. Found: C, 57.91; H, 4.39; N, 5.39. $^{1H\ NMR\ (DMSO\text{-}d_6)}$ δ: 3.16 (s, 3H, 5-CH_3), 4.02 (s, 3H, 6-CH_3), 4.21 (s, 3H, 2-CH_3), 7.12 (m, 3H, 8-H+ Phenyl-H), 7.46 (d, J_{10-8} = 2.3 Hz, 1H, 10-H), 7.51 (d, J = 8.5 Hz, 2H, Phenyl-H), 7.57 (d, J_{7-8} = 8.8 Hz, 1H, 7-H), 8.17 (s, 1H, 11-H), 8.60 (m, 2H, 3-H+ 4-H), 9.33 (s, 1H, 4'-OH), 10.34 (s, 1H, 9-OH).

13d: Yield 95%; mp 297-298°C (from ethanol). Anal. calcd. for $C_{26}H_{25}N_2O_2I$: C, 59.55; H, 4.81; N, 5.34. Found: C, 59.23; H, 4.97; N, 5.17. ^{1}H NMR (DMSO-d_6) δ: 3.18 (s, 3H, 5-CH_3), 3.81 (s, 3H, 4'-OCH_3), 3.94 (s, 3H, 9-OCH_3), 4.05 (s, 3H, 6-CH_3), 4.20 (s, 3H, 2-CH_3), 7.28 (dd, J_{8-7}= 8.9 Hz, J_{8-10} = 2.5 Hz, 1H, 8-H), 7.33 (d, J = 8.7 Hz, 2H, Phenyl-H), 7.65 (m, 3H, 7-H+ Phenyl-H), 7.87 (d, J_{10-8} = 2.4 Hz, 1H, 10-H), 8.38 (s, 1H, 11-H), 8.55 (d, J_{4-3} = 7.4 Hz, 1H, 4-H), 8.62 (d, 1H, 3-H).

Biological test procedures

Human tumor cell lines

Test solutions of all the tested compounds **5c** and **11a**−**13d** (1 mg/mL) were prepared *ex tempore* for each test by dissolving them in 100 μL of DMSO + 900 μL of culture medium. After that, the compounds were diluted in culture medium (described below) to reach the final concentrations of 100, 10, 1, and 0.1 μg/mL.

Cell lines

Cells of the human A549 cell line (non-small cell lung cancer) were used. This line was obtained from American Type Culture Collection (Rockville, MD, USA) and cultured at the Cell Culture Collection of the Department of Tumor Immunology, Institute of Immunology and Experimental Therapy, Wrocław, Poland. 24 h before adding the tested agents, the cells were plated in 96-well plates (Sarstedt, Newton, NC, USA) at a density of 10^4 cells per well. The cells were cultivated in opti-MEM medium supplemented with 2 mM glutamine (Gibco, Warsaw, Poland), streptomycin (50 μg/mL), penicillin (50 U/mL) (both antibiotics from Polfa, Tarchomin, Poland), and 5% fetal calf serum (Gibco, Grand Island, USA). The cell cultures were maintained at 37°C in a humid atmosphere with 5% CO_2.

SRB assay

The cytotoxicity assays were performed after 72-h exposure of the cultured cells to varying concentrations (from 0.1 to 100 μg/mL) of the tested agents. The SRB method was used as described by Skehan et al. [12]. The optical densities of the samples were measured on a Multiskan RC photometer (Labsystems, Helsinki, Finland) at 570 nm.

The results were expressed as IC_{50} values (inhibitory concentration 50%), the dose of the compound which inhibits the proliferation rate of the tumor cells by 50% compared with untreated control cells, and these are summarized in Table 1 in comparison to compound **5c**. Each compound was tested in triplicate at every concentration. Each experiment was repeated 3 times.

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