Full Paper

Synthesis and Anticancer Activity of New 1-Substituted-6*H*-pyrido[4,3-*b*]carbazole Derivatives

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This study examines the synthesis and cytostatic activity of new 5,6-dimethyl-1-substituted-6*H*-pyrido[4,3-*b*]carbazole derivatives. Their structures were confirmed by ^{1}H-NMR and elemental analysis. Seven of the new compounds were tested by the SRB method *in vitro* against human lung cancer (A549) and human kidney cancer (A498) cell lines. Biological tests indicated remarkable cytostatic effects of four compounds tested in comparison with ellipticine and cisplatin as reference drugs. One particular compound **3c** was about four times more active on A498 than ellipticine with similar activity on the A549 cell line, and outperformed cisplatin activity on both tumor cell lines.

Keywords: Cytostaticity / Olivacine / Pyridocarbazole

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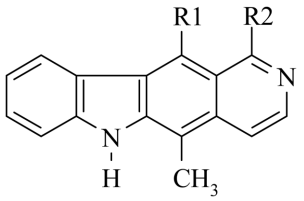
Introduction

Olivacine **1** and ellipticine **2** (Fig. 1.) are natural alkaloids isolated from *Aspidosperma olivaceum* Müll. Arg. [1] and *Ochrosia elliptica* [2] from the *Apocynacea* family.

The main reason for the interest in these compounds is their anticancer activity [3, 4]. Although still unclear, their mechanism of action is considered to be mainly based on DNA intercalation and/or inhibition of topoisomerase II [5]. Based on naturally occurring lead compounds, numerous modifications of their heterocyclic ring system have been studied [6] and lead to the development of drugs like *elliptinium* [7 – 9], *datelliptium* [10], *retelliptine* [11] or *pazaelliptine* [12].

Until now, much less attention has been paid to the synthesis of analogues of the olivacine **1**, which has also been proved to possess potential anticancer properties. One of the olivacine derivatives namely 9-hydroxy-5,6-dimethyl-*N*-[2-(dimethylamino)ethyl]-6*H*-pyrido[4,3-*b*]carbazole-1-carboxamide (S 16020) is actually in the course of clinical trials [13, 14]. S 16020 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 models [15 – 17]. Other olivacine analogues, 1-pyridylsubstituted pyrido[4,3-*b*]carbazole derivatives have also shown strong cytostatic activity when tested *in vitro*.



Olivacine **1** R_1=H, R_2=CH_3
Ellipticine **2** R_1=CH_3, R_2=H

Figure 1. Structures of natural alkaloids olivacine **1** and ellipticine **2**.

According to the previously obtained results [18 – 20], the sole attempt to synthesize 1-phenyl-6*H*-pyrido[4,3-*b*]carbazole derivatives was undertaken. One of the newly obtained compounds, namely 5,6-dimethyl-9-hydroxy-1-

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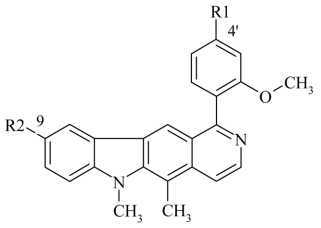


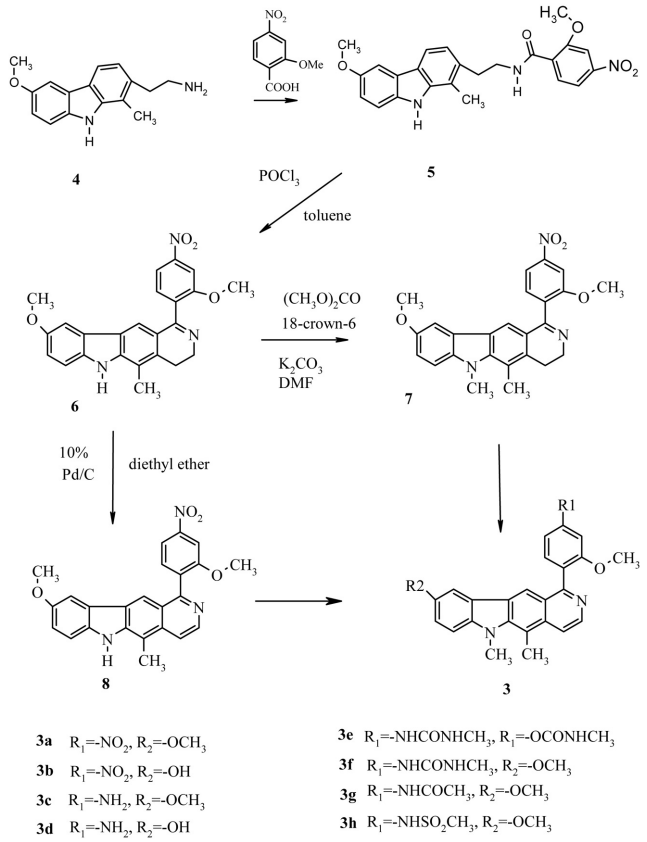
3

3a R_1=-NO_2, R_2=-OCH_3
3b R_1=-NO_2, R_2=-OH
3c R_1=-NH_2, R_2=-OCH_3
3d R_1=-NH_2, R_2=-OH
3e R_1=-$NHCONHCH_3$, R_1=-$OCONHCH_3$
3f R_1=-$NHCONHCH_3$, R_2=-OCH_3
3g R_1=-$NHCOCH_3$, R_2=-OCH_3
3h R_1=-$NHSO_2CH_3$, R_2=-OCH_3

Figure 2. Structures of the newly obtained pyrido[4,3-*b*]carbazole derivatives **3**.

(4-nitrophenyl)-6*H*-pyrido[4,3-*b*]carbazole has shown significant antitumor activity on human lung cancer (A549) (IC_{50} = 2.37 μM) [21]. This fact prompted us to synthesize new 1-phenyl-2′-methoxy-6*H*-pyrido[4,3-*b*]carbazole derivatives **3**, which were subjected for testing their cytostatic activity *in vitro* on human lung cancer (A549) and human kidney cancer (A498) cell lines.

These pyrido[4,3-*b*]carbazole derivatives (Fig. 2) were obtained according to Scheme 1.



3a R_1=-NO_2, R_2=-OCH_3
3b R_1=-NO_2, R_2=-OH
3c R_1=-NH_2, R_2=-OCH_3
3d R_1=-NH_2, R_2=-OH
3e R_1=-$NHCONHCH_3$, R_1=-$OCONHCH_3$
3f R_1=-$NHCONHCH_3$, R_2=-OCH_3
3g R_1=-$NHCOCH_3$, R_2=-OCH_3
3h R_1=-$NHSO_2CH_3$, R_2=-OCH_3

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

Results and discussion

2.1 Synthesis and chemistry

The starting compound 2-(6-methoxy-1-methyl-9*H*-carbazol-2-yl)ethylamine **4** has been previously described [2]. It was allowed to react with 2-methoxy-4-nitrobenzoic acid using the mixed anhydride method. Cyclization of the resulting amide **5** with phosphorous oxychloride in boiling toluene gave 9-methoxy-5-methyl-1-(2′-methoxy-4′-nitrophenyl)-3,4-dihydro-6*H*-pyrido[4,3-*b*]carbazole **6**, which was aromatized to derivative **8** by dehydrogenation over 10% palladium on charcoal in boiling diphenyl ether. The same protocol for the aromatization of **7** was used and led to 5,6-dimethyl-9-methoxy-1-(2′-methoxy-4′-nitrophenyl)-6*H*-pyrido[4,3-*b*]carbazole **3a**. *N*-6-methylation of **6** to **7** and **8** to **3a** were performed using an excess of dimethyl carbonate in dimethylformamide in the presence of potassium carbonate and 18-crown-6. The compound **3a** was 9-*O*-demethylated to derivative **3b** by reaction with boron tribromide at −70°C. The compound **3c** was obtained by heating 5,6-dimethyl-9-methoxy-1-(2′-methoxy-4′-nitrophenyl)-6*H*-pyrido[4,3-b]carbazole **4a** with 10% palladium on charcoal in glacial acetic acid. Demethylation of the amine **3c** by heating with hydrobromic acid gave 5,6-dimethyl-9-hydroxy-1-(4′-amino-2′-methoxyphenyl)-6*H*-pyrido[4,3-*b*]carbazole **3d**, which was mixed with methyl isocyanate in dry chloroform to form derivative **3e**. The same protocol was used for **3c** and led to the derivative **3f**. Compound **3g** was obtained by reaction of 5,6-dimethyl-9-methoxy-1-(4′-amino-2′-methoxyphenyl)-6*H*-pyrido[4,3-*b*]carbazole **3c** with an acetic anhydride : pyridine mixture (1 : 1) at room temperature. The 5,6-dimethyl-9-methoxy-1-(4′-methanesulfamoyl-2′-methoxyphenyl)-6*H*-pyrido[4,3-*b*]carbazole **3h** was prepared reacting compound **3c** with methanesulfonyl chloride in chloroform.

Biology

Seven of the new 1-substituted-6*H*-pyrido[4,3-*b*]carbazole derivatives **3b**–**3h** were subjected to preliminary *in-vitro* cytostatic activity screening. They exhibited different biological properties depending on the kind of substituent at the 9- and 4′-positions of the ring system. All screening data are shown in Table 1. Compound **3b** exhibited lower cytostatic activity than the reference drugs on both tumor cell lines tested. Considerable increase in activity was caused by the reduction of the 4′-nitro group of compound **3b** to a 4′-amino group resulting in compounds **3c**

Table 1. Cell growth inhibition on A498 (kidney cancer) and A549 (non-small-cell lung cancer) cells. IC_{50} values (μM) ± *SD* of compounds **3b – 3h** compared to ellipticine and cisplatin.

Compound	A498 (μM)	A549 (μM)
3b	5.84 ± 0.482	4.35 ± 0.460
3c	0.437 ± 0.382	0.867 ± 0.060
3d	0.766 ± 0.052	0.808 ± 0.046
3e	5.32 ± 0.405	inactive
3f	0.822 ± 0.041	0.924 ± 0.061
3g	10.76 ± 3.83	8.78 ± 1.72
3h	0.700 ± 0.060	1.24 ± 0.323
Cisplatin	**1.18 ± 0.110**	**1.32 ± 0.281**
Ellipticine	**1.74 ± 0.040**	**0.85 ± 0.040**

and **3d**. Moreover, it can be noticed that 9-*O*-demethylation of derivative **3c** decreased the cytostatic activity against A498 cells as revealed from the higher IC_{50} value of compound **3d** (from 0.437 μM to 0.766 μM). The *N*-acetylation of derivative **3c** to compound **3g** resulted in a drop of cytostatic properties (from 0.437 μM to 10.76 μM). However, the substitution of amino group with methylsulfonyl or methylcarbamoyl moieties led to only slightly less active compounds **3h** and **3f**, respectively. In addition, a drop of antiproliferative activity was also observed due to the substitution of the hydroxyl group at the 9-position with a methylcarbamoyl unit resulting in compound **3e,** having poor cytostatic properties in comparison with its 9-*O*-methylated structural analogue **3f**.

Conclusions

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

Two new pyridocarbazole derivatives **3c** and **3d** with a primary amino group at the 4′-position were significantly more active on the A498 cell line than both reference drugs. Their cytostatic activities on A549 cell line were comparable with ellipticine and better than those of cisplatin. The antiproliferative properties of compounds **3f** and **3h** on the A498 cell line also outperformed both of the reference drugs, but their activity on the A549 cell line were comparable with cisplatin and slightly less than those of ellipticine. Compounds **3b**, **3e**, and **3g** exhibited significantly lower cytostatic effect in comparison to the reference drugs on both tumor cell lines.

Pharmacological results suggest the presence of an unsubstituted amino group at the 4′-position to be the most advantageous for the antiproliferative activity of the tested compounds. Its acetylation resulted in an activity drop while substitution with methylsulfonyl or methylcarbamoyl groups led to only slightly less active compounds. It can be also noticed that the substitution of the hydroxyl group at the 9-position with methylcarbamoyl moiety led to a significant lowering of the cytostatic properties. These facts suggest that it will be interesting to synthesize a series of compounds with *N*-alkyl- and *N*,*N*-dialkylamino moieties at the 4′-position of 1-phenyl-substituted-6*H*-pyrido[4,3-*b*]carbazole ring system to test the influence of alkyl groups on the cytostatic properties of this class of compounds.

The authors have declared no conflict of interest.

Experimental

Melting points were determined on a Köffler apparatus (C. Reichert, Vienna, Austria) 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 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 compound 2-(6-methoxy-1-methyl-9*H*-carbazol-2-yl)ethylamine **4** (Scheme 1) was prepared according to the described procedure [13].

Chemistry and Synthesis

*N-[2-(6-Methoxy-1-methyl-9H-carbazole)ethyl]-2-methoxy-4-nitro-1-phenylcarboxamide **5***

Triethylamine (0.75 g, 7.5 mmol) was added to 2-methoxy-4-nitrobenzoic acid (1.3 g, 6.6 mmol) dissolved in dry tetrahydrofuran. After cooling to −10°C, a solution of ethyl chloroformate (0.81 g, 7.5 mmol) in dry THF (20 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** (1.52 g, 6 mmol) in tetrahydrofuran (100 mL) was added dropwise at −10°C. The resulting mixture was left for 20 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 methylene chloride, and then dried over magnesium sulfate. Evaporation of the solvent provided a solid residue, which was recrystallized from ethanol to yield 2.3 g (90%) of compound **5**, mp.: 227 – 229°C. Anal. calcd. for $C_{24}H_{23}N_3O_5$: C, 66.50; H, 5.35; N, 9.69. Found: C, 66.32; H, 5.54; N, 9.46. ^{1}H-NMR (DMSO-d_6) δ: 2.52 (s, 3H, 1-CH_3), 2.99 (m, 2H, α-CH_2), 3.49 (m, 2H, β-CH_2), 3.82 (s, 3H, 2′-OCH_3), 3.92 (s, 3H, 6-OCH_3), 6.94 (m, 2H, 7-H, 3-H), 7.36 (d, $J_{8\text{-}7}$ = 8.7 Hz, 1H, 8-H), 7.59 (d, $J_{5\text{-}8}$ = 2.4 Hz, 1H, 5-H), 7.89 (m, 4H, 3′-H, 5′-H, 6′-H, 4-H), 8.48 (m, 1H, -NHCO), 10.85 (s, 1H, 9-NH).

*9-Methoxy-5-methyl-1-(2′-methoxy-4′-nitro)phenyl-3,4-dihydro-6H-pyrido[4,3-b]carbazole **6***

The preceding amide **5** (2.16 g, 5 mmol) was dissolved in boiling toluene (250 mL) and then treated dropwise with phosphorous

oxychloride (20 mL). The reflux was continued for 12 h and evaporation under reduced pressure afforded a residue which was taken up in water (150 mL), alkalized to pH 9–10 with conc. aq. ammonia, and extracted with methylene chloride. The extract was dried over magnesium sulfate. After evaporation of the solvent, the solid was purified by column chromatography on silica gel and eluted with methylene chloride/methanol, 97 : 3, v/v. The pure base was obtained by evaporating the fractions containing the expected product. Yield: 88%; mp.: 247–248°C. Anal. calcd. for $C_{24}H_{21}N_3O_4$: C, 69.39; H 5.10; N 10.11. Found: C, 69.17; H 5.26; N 9.97. ^{1}H-NMR (DMSO-d_6) δ: 2.71 (s, 3H, 5-CH_3), 2.93 (m, 2H, 4-CH_2), 3.74 (m, 6H, 2′-OCH_3, 9-OCH_3), 3.93 (m, 2H, 3-CH_2), 6.96 (dd, $J_{8\text{-}7}$ = 8.7 Hz, $J_{8\text{-}10}$ = 2.4 Hz, 1H, 8-H), 7.37 (d, $J_{7\text{-}8}$ = 8.7 Hz, 1H, 7-H), 7.43 (s, 1H, 11-H), 7.47 (d, $J_{10\text{-}8}$ = 2.4 Hz, 1H, 10-H), 7.57 (d, $J_{6'\text{-}5'}$ = 8.2 Hz, 1H, 6′-H), 7.91 (d, $J_{3'\text{-}5'}$ = 2.1 Hz, 1H, 3′-H), 7.98 (dd, $J_{5'\text{-}3'}$ = 2.1 Hz, $J_{5'\text{-}6'}$ = 8.2 Hz, 1H, 5′-H), 11.21 (s, 1H, 6-H).

5,6-Dimethyl-9-methoxy-1-(2'-methoxy-4'-nitro)phenyl-3,4-dihydro-6H-pyrido[4,3-b]carbazole ***7***

A mixture of compound **6** (0.82 g, 2 mmol), dry potassium carbonate (0.5 g), dimethyl carbonate (20 mL), dimethylformamide (2 mL), and a trace of 18-crown-6 was refluxed with stirring for 12 h. After evaporation to dryness, the residue was taken up in water, alkalized with conc. aq. ammonia, and extracted with methylene chloride. The extract was then 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 methylene chloride to give 0.35 g (41%) of compound **7**, mp. 249°C. Anal. calcd. for $C_{25}H_{23}N_3O_4$: C, 69.92; H, 5.40; N, 9.78. Fund: C, 70.05; H, 5.55; N, 9.52. ^{1}H-NMR (DMSO-d_6) δ: 2.74 (s, 3H, 5-CH_3), 2.88 (m, 2H, 4-CH_2), 3.76 (m, 6H, 2′-OCH_3, 9-OCH_3), 3.96 (m, 2H, 3-CH_2), 4.09 (s, 3H, 6-CH_3), 7.02 (dd, $J_{8\text{-}7}$ = 8.8 Hz, $J_{8\text{-}10}$ = 2.4 Hz, 1H, 8-H), 7.47 (m, 3H, 7-H, 10-H, 11-H), 7.58 (d, $J_{6'\text{-}5'}$ = 8.2 Hz, 1H, 6′-H), 7.90 (d, $J_{3'\text{-}5'}$ = 2.1 Hz, 1H, 3′-H), 7.98 (dd, $J_{5'\text{-}3'}$ = 2.1 Hz, $J_{5'\text{-}6'}$ = 8.2 Hz, 1H, 5′-H).

9-Methoxy-5-methyl-1-(2'-methoxy-4'-nitro)phenyl-6H-pyrido[4,3-b]carbazole ***8***

Compound **6** (2.07 g, 5 mmol) was refluxed in diphenyl ether (50 mL) in the presence of 10% palladium on charcoal (0.30 g) for 3 h. The catalyst was filtered off and the filtrate was cooled and diluted with 100 mL of hexane. The resulting precipitate was collected, washed with hexane, and purified by chromatography on silica gel column and eluted with methylene chloride to give 0.95 g (46%) of **8**; mp.: 282°C. Anal. calcd. for $C_{24}H_{19}N_3O_4$: C, 69.72; H, 4.63; N, 10.16. Found: C, 69.47; H, 4.78; N, 10.40. ^{1}H-NMR (DMSO-d_6) δ: 2.84 (s, 3H, 5-CH_3), 3.78 (s, 3H, 2′-OCH_3), 3.79 (s, 3H, 9-OCH_3), 7.11 (dd, $J_{8\text{-}7}$ = 8.7 Hz, $J_{8\text{-}10}$ = 2.4 Hz, 1H, 8-H), 7.42 (d, $J_{7\text{-}8}$ = 8.7 Hz, 1H, 7-H), 7.65 (d, $J_{6'\text{-}5'}$ = 8.0 Hz, 1H, 6′-H), 7.77 (d, $J_{10\text{-}8}$ = 2.4 Hz, 1H, 10-H), 7.99 (d, $J_{4\text{-}3}$ = 6.2 Hz, 1H, 4-H), 8.04 (m, 2H, 3′-H, 5′-H), 8.20 (s, 1H, 11-H), 8.45 (d, $J_{3\text{-}4}$ = 6.2 Hz, 1H, 3-H).

5,6-Dimethyl-9-methoxy-1-(2'-methoxy-4'-nitro)phenyl-6H-pyrido[4,3-b]carbazole ***3a***

Method A: Compound **3a** was synthesized using a similar procedure described for **7**, starting from **8**. Yield: 84%; mp.: 275–276°C. Anal. calcd. for $C_{25}H_{21}N_3O_4$: C, 70.25; H, 4.95; N, 9.83. Found: C, 70.10; H, 5.10; N, 9.51. ^{1}H-NMR (DMSO-d_6) δ: 3.09 (s, 3H, 5-CH_3), 3.77 (s, 3H, 2′-OCH_3) 3.80 (s, 3H, 9-OCH_3), 4.13 (s, 3H, 6-CH_3), 7.16 (dd, $J_{8\text{-}7}$ = 8.8 Hz, $J_{8\text{-}10}$ = 2.4 Hz, 1H, 8-H), 7.51 (d, $J_{7\text{-}8}$ = 8.9 Hz, 1H, 7-H), 7.66 (d, $J_{5'\text{-}6'}$ = 7.9 Hz, 1H, 6′-H), 7.77 (d, $J_{10\text{-}8}$ = 2.4 Hz, 1H, 10-H), 8.02 (s, 1H, 3′-H), 8.04 (d, $J_{5'\text{-}6'}$ = 8.1 Hz, 1H, 5′-H), 8.07 (d, $J_{4\text{-}3}$ = 6.2 Hz, 1H, 4-H), 8.21 (s, 1H, 11-H), 8.46 (d, $J_{3\text{-}4}$ = 6.2 Hz, 1H, 3-H).

Method B: Compound **7** was aromatized to derivative **3a** using a procedure described for compound **8**. Yield: 50%; mp.: 275°C.

5,6-Dimethyl-9-hydroxy-1-(2'-methoxy-4'-nitro)phenyl-6H-pyrido[4,3-b]carbazole ***3b***

Compound **3a** (0.21 g, 0.5 mmol) was dissolved in 100 mL of methylene chloride and boron tribromide (10 mL) was added dropwise at −70°C. The reaction mixture was stirred under nitrogen at normal pressure for 2.5 h, maintaining a temperature of −70°C. Then, the mixture was stirred at room temperature for 12 h and evaporated to dryness. The residue was taken up in water (50 mL), basified with conc. aq. ammonia, extracted with methylene chloride, and then dried over magnesium sulfate. After evaporation of the solvent, the solid was purified by chromatography on a silica gel column and eluted with methylene chloride/methanol, 99 : 1, v/v. By evaporation of the fractions containing the expected product, the pure base was thus obtained. Yield: 13%; mp.: 160–161°C. Anal. calcd. for $C_{24}H_{19}N_3O_4$: C, 69.72; H, 4.63; N, 10.16. Found: C, 69.44; H, 5.34; N, 9.95. ^{1}H-NMR (DMSO-d_6) δ: 3.07 (s, 3H, 5-CH_3), 3.76 (s, 3H, 2′-OCH_3), 4.09 (s, 3H, 6-CH_3), 7.01 (dd, $J_{8\text{-}7}$ = 8.7 Hz, $J_{8\text{-}10}$ = 2.3 Hz, 1H, 8-H), 7.40 (d, $J_{7\text{-}8}$ = 8.7 Hz, 1H, 7-H), 7.45 (d, $J_{10\text{-}8}$ = 2.3 Hz, 1H, 10-H), 7.62 (m, 1H, 6-H), 7.86 (m, 2H, 3′-H, 5′-H), 8.04 (d, $J_{4\text{-}3}$ = 6.2 Hz, 1H, 4-H), 8.14 (s, 1H, 11-H), 8.45 (d, $J_{3\text{-}4}$ = 6.2 Hz, 1H, 3-H), 9.10 (s, 1H, 9-OH).

5,6-Dimethyl-9-methoxy-1-(4'-amino-2'-methoxy)phenyl-6H-pyrido[4,3-b]carbazole ***3c***

Compound **3a** (0.85 g, 2 mmol) was dissolved in 150 mL of glacial acetic acid and 10% palladium on charcoal (80 mg) was added. The mixture was heated to 50°C and maintained at this temperature under hydrogen at normal pressure for 1h. The catalyst was filtered off, the solvent was evaporated to dryness, and the solid was purified by column chromatography on silica gel and eluted with methylene chloride/methanol, 97 : 3, v/v. By evaporating the fractions containing the expected product, the pure base was obtained. Yield: 98%; mp.: 143–145°C. Anal. calcd. for $C_{25}H_{23}N_3O_2$: C, 75.55; H, 5.83; N, 10.57. Found: C, 75.37; H, 6.01; N, 10.34. ^{1}H-NMR (DMSO-d_6) δ: 3.05 (s, 3H, 5-CH_3), 3.54 (s, 3H, 2′-OCH_3) 3.82 (s, 3H, 9-OCH_3), 4.10 (s, 3H, 6-CH_3), 5.39 (s, 2H, 4′-NH_2), 6.33 (dd, $J_{5'\text{-}3'}$ = 1.8 Hz, $J_{5'\text{-}6'}$ = 8.0 Hz , 1H, 5′-H), 6.42 (d, $J_{3'\text{-}5'}$ = 1.8 Hz, 1H, 3′-H), 7.04 (d, $J_{6'\text{-}5'}$ = 8.0 Hz, 1H, 6′-H), 7.14 (dd, $J_{8\text{-}7}$ = 8.8 Hz, $J_{8\text{-}10}$ = 2.4 Hz, 1H, 8-H), 7.49 (d, $J_{7\text{-}8}$ = 8.8 Hz, 1H, 7-H), 7.58 (d, $J_{10\text{-}8}$ = 2.4 Hz, 1H, 10-H), 7.89 (d, $J_{4\text{-}3}$ = 6.2 Hz, 1H, 4-H), 8.30 (s, 1H, 11-H), 8.37 (d, $J_{3\text{-}4}$ = 6.2 Hz, 1H, 3-H).

5,6-Dimethyl-9-hydroxy-1-(4'-amino-2'-methoxy)phenyl-6H-pyrido[4,3-b]carbazole ***3d***

A mixture of compound **3c** (0.158 g, 0.4 mmol) and hydrobromic acid (40 mL) was heated under reflux 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 evaporating the solvent, the solid residue was purified by chromatography on a silica gel column and eluted with methylene chloride/methanol,

97 : 3, v/v. Yield: 35%; mp.: >300°C. Anal. calcd. for $C_{24}H_{21}N_3O_2$: C, 75.18; H, 5.52; N, 10.96. Found: C, 74.97; H, 5.71; N, 10.87. ^{1}H-NMR (DMSO-d_6) δ: 3.04 (s, 3H, 5-CH_3), 3.53 (s, 3H, 2′-OCH_3), 4.08 (s, 3H, 6-CH_3), 5,42 (s, 2H, 4′-NH_2) 6.34 (dd, $J_{5'-3'}$ = 1.7 Hz, $J_{5'-6'}$ = 8.0 Hz, 1H, 5′-H), 6.43 (d, $J_{3'-5'}$ = 1.7 Hz, 1H, 3′-H), 6.99 (dd, J_{8-7} = 8.7 Hz, J_{8-10} = 2.3 Hz, 1H, 8-H), 7.03 (d, $J_{6'-5'}$ = 8.0 Hz, 1H, 6'-H), 7.36 (d, J_{10-8} = 2.2 Hz, 1H, 10-H), 7.40 (d, J_{7-8} = 8.7 Hz, 1H, 7-H), 7.90 (d, J_{4-3} = 6.3 Hz, 1H, 4-H), 8.14 (s, 1H, 11-H), 8.35 (d, J_{3-4} = 6.3 Hz, 1H, 3-H), 9.08 (s, 1H, 9-OH).

5,6-Dimethyl-9-methylcarbamoyloxy-1-[2′-methoxy-4′-(3-methylureilene)]phenyl-6H-pyrido[4,3-b]carbazole ***3e***

A mixture of **3d** (0.191 g, 0.5 mmol), 4-dimethylaminopyridine (70 mg) and dry chloroform (50 mL) was stirred for 0.5 h and then methyl isocyanate (5 mL) was added. The mixture was stirred at room temperature for 24 h. After this time, water was added and the mixture was extracted with chloroform; organic layers were collected, dried, and evaporated to dryness under reduced pressure. The solid residue was purified using column chromatography with silica gel and eluted with dichloromethane/ methanol, 99 : 1, v/v. Yield: 22%; mp.: 158 – 159°C. Anal. calcd. for $C_{28}H_{27}N_5O_4$: C, 67.59; H, 5.47; N, 14.08. Found: C, 67.27; H, 5.62; N, 13.89. ^{1}H-NMR (DMSO-d_6) δ: 2.67 (s, 2 × 3H, -$NHCH_3$), 3.10 (s, 3H, 5-CH_3), 3.42 (s, 3H, 2′-OCH_3), 4.17 (s, 3H, 6-CH_3), 6.13 (s, 1H, 9-COONH), 7.04 (s, 1H, 3′-H), 7.31 (m, 3H, 8-H, 6′-H, 5′-H), 7.54 (s, 1H, 10-H), 7.58 (s, 1H, 7-H), 7.77 (s, 1H, 4′-$CONHCH_3$), 8.01 (d, J_{4-3} = 6.1 Hz, 1H, 4-H), 8.40 (s, 1H, 11-H), 8.44 (d, J_{4-3} = 6.1 Hz, 1H, 3-H), 8.85 (s, 1H, 4′-NHCO).

5,6-Dimethyl-9-methoxy-1-[2′-methoxy-4′-(3-methylureilene)]phenyl-6H-pyrido[4,3-b]carbazole ***3f***

The compound was synthesized using a similar procedure as described for compound **3e** starting from **3c**. Yield: 35%; mp.: 290°C. Anal. calcd. for $C_{27}H_{26}N_4O_3$: C, 71.35; H, 5.77; N, 12.33. Found: C, 71.23; H, 5.93; N, 12.12. ^{1}H-NMR (DMSO-d_6) δ: 2.69 (d, $J_{NHCH3-NHCH3}$ = 4.6 Hz, 3H, -$NHCH_3$), 3.08 (s, 3H, 5-CH_3), 3.58 (s, 3H, 2′-OCH_3), 3.81 (s, 3H, 9-OCH_3), 4.13 (s, 3H, 6-CH_3), 6.11 (d, $J_{NHCH3-NHCH3}$ = 4.6 Hz, 1H, -$NHCH_3$), 7.09 (d, $J_{5'-6'}$ = 8.1 Hz, 1H, 5′-H), 7.14 (dd, J_{8-7} = 8.8 Hz, J_{8-10} = 2.4 Hz, 1H, 8-H), 7.21 (d, $J_{6'-5'}$ = 8.1 Hz, 1H, 6′-H), 7.47 (s, 1H, 3′-H), 7.51 (d, J_{7-8} = 8.8 Hz, 1H, 7-H), 7.61 (d, J_{10-8} = 2.4 Hz, 1H, 10-H), 7.96 (d, J_{4-3} = 6.2 Hz, 1H, 4-H), 8.25 (s, 1H, 11-H), 8.40 (d, J_{3-4} = 6.2 Hz, 1H, 3-H), 8.78 (s, 1H, 4′-NH).

5,6-Dimethyl-9-methoxy-1-(4′-acetamido-2′-methoxy)phenyl-6H-pyrido[4,3-b]carbazole ***3g***

Compound **3c** (0.19 g, 0.5 mmol) was dissolved in 20 mL of acetic anhydride/pyridine (1 : 1). The mixture was stirred at room temperature for 2 h and evaporated to dryness. The solid was purified by column chromatography on silica gel and eluted with methylene chloride. By evaporating the fractions containing the expected product, the pure base was obtained. Yield: 82%; mp.: 218 – 219°C. Anal. calcd. for $C_{27}H_{25}N_3O_3$: C, 73.79; H, 5.73; N, 9.56. Found: C, 73.57; H, 6.01; N, 9.39. ^{1}H-NMR (DMSO-d_6) δ: 2.11 (s, 3H, -$COCH_3$), 3.07 (s, 3H, 5-CH_3), 3.59 (s, 3H, 2′-OCH_3), 3.81 (s, 3H, 9-OCH_3), 4.12 (s, 3H, 6-CH_3), 7.14 (dd, J_{8-7} = 8.8 Hz, J_{8-10} = 2.2 Hz, 1H, 8-H), 7.28 (d, $J_{5'-6'}$ = 8.1 Hz , 1H, 5′-H), 7.35 (d, $J_{6'-5'}$ = 8.2 Hz, 1H, 6′-H), 7.50 (d, J_{7-8} = 8.8 Hz, 1H, 7-H), 7.59 (s, 1H, 3′-H), 7.63 (d, J_{10-8} = 2.2 Hz, 1H, 10-H), 7.97 (d, J_{4-3} = 6.2 Hz, 1H, 4-H), 8.23 (s, 1H, 11-H), 8.41 (d, J_{3-4} = 6.2 Hz, 1H, 3-H), 10.17 (s, 1H, 4′-HCO).

5,6-Dimethyl-9-methoxy-1-(4′-methanesulfamoyl-2′-methoxy)phenyl-6H-pyrido[4,3-b]carbazole ***3h***

Compound **3c** (0.5 mmol) and 4-dimethylaminopyridine (70 mg) was dissolved in chloroform (50 mL). Then methanesulfonyl chloride (1 mL) was added after 30 min with stirring and maintained at room temperature for 24 h. The reaction mixture was evaporated under reduced pressure and the solid residue was purified using column chromatography with neutral alumina grade II and dichloromethane as eluent. Yield: 84%; mp.: 297 – 299°C. Anal. calcd. for $C_{26}H_{25}N_3O_4S$: C, 65.67; H, 5.30; N, 8.84. Found: C, 65.44; H, 5.46; N, 8.67. ^{1}H-NMR (DMSO-d_6) δ: 3.07 (s, 3H, SO_2CH_3), 3.15 (s, 3H, 5-CH_3), 3.60 (s, 3H, 2′-OCH_3), 3.81 (s, 3H, 9-OCH_3), 4.12 (s, 3H, 6-CH_3), 7.01 (d, $J_{5'-6'}$ = 8.1 Hz, 1H, 5′-H), 7.07 (s, 1H, 3′-H), 7.16 (dd, J_{8-7} = 8.7 Hz, J_{8-10} = 2.2 Hz, 1H, 8-H), 7.33 (d, $J_{6'-5'}$ = 8.0 Hz, 1H, 6′-H), 7,50 (d, J_{7-8} = 8.8 Hz, 1H, 7-H), 7.66 (d, J_{10-8} = 2.3 Hz, 1H, 10-H), 7.98 (d, J_{4-3} = 6,2 Hz, 1H, 4-H), 8.25 (s, 1H, 11-H), 8.41 (d, J_{3-4} = 6.3 Hz, 1H, 3-H), 9.99 (s, 1H, 4′-NH).

Biological testing

Test solutions of seven 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 0.0001 μg/mL.

Cell lines

The human cancer cell lines A498 (kidney cancer) and A549 (non-small-cell lung cancer) were used. Both lines were cultured in the Cell Culture Collection of the Department of Tumor Immunology, Institute of Immunology and Experimental Therapy, Wroclaw, Poland.

The A549 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). The A498 cells were cultivated in Eagle medium supplemented with 10% serum (FCS), 1 mM sodium pyruvate, 2 mM glutamine, 100 U/mL penicillin and 100 μg/mL streptomycin. The cell cultures were maintained at 37°C in a humid atmosphere containing 5% CO_2.

SRB

The SRB method was used as described by Skehan *et al*. [22]. The cytostatic assays were performed after 72-hour exposure of the cultured cells to varying concentrations of the tested agents. Each experiment was repeated three times. The 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 tested compounds.

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