DRUG SYNTHESIS

DESIGN, SYNTHESIS AND ANALYSIS OF ANTICANCER ACTIVITY OF NEW SAR-BASED S16020 DERIVATIVES

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Abstract: A novel set of S16020 derivatives was designed applying structure-activity relationships (SAR). The newly synthesized compounds were subjected to *in vitro* cytostatic activity screening against human kidney cancer (cell line A498), human lung cancer (cell line A549) and normal human dermal fibroblasts (cell line NHDF). Two 6*H*-pyrido[4,3-*b*]carbazole derivatives exhibited stronger potency against both investigated cell lines than the reference compounds ellipticine and doxorubicin.

Keywords: olivacine, cytostatic, A498, A549, NHDF, S16020

Structure-activity relationships (SAR) is a method based on a simple and fundamental discovery that the biological or physicochemical activity of a chemical compound relates to its structure. Hence the simple assumption that similar molecules have similar activities and minor modifications (e.g. replacement of substituents, magnification of the molecule, ring condensation etc.) to the molecular structure of the active substance could yield compounds with even better activity or selectivity, and/or improved pharmacokinetic properties (1-3). Pyridocarbazole- based compounds, like ellipticine

and structurally connected olivacine, have shown significant biological activity. One of the pyridocarbazole-based derivatives 9-hydroxy-5,6-dimethyl-*N*-[2-(dimethylamino)ethyl]-6*H*-pyrido[4,3-*b*]carbazole-1-carboxamide (S16020) (Fig. 1) has displayed impressive cytotoxic potency (4-5). The compound has shown marked antitumor activity against human (lung, breast, colon, and ovary) and murine (B16 melanoma, P388 leukemia, Lewis lung carcinoma, M5076 sarcoma) tumor models (6-12). Importantly, S 16020 has already been tested in a few clinical trials (13, 14) and confirms suggestions that the most active compounds act as DNA intercalators. One of the new series of 6*H*-pyrido[4,3-*b*]carbazole derivatives: 9-hydroxy-5,6-dimethyl-*N*-[2-(dimethylamino)ethyl]-6*H*-pyrido[4,3-*b*]carbazole-1-carboxamide **1** is an intercalating compound exhibiting a high DNA binding affinity and inhibition of topoisomerase II, which is responsible for its pharmacological activity (8, 15). Using literature data and based on our results (16-20) a new set of new pyrido[4,3-*b*]carbazole derivatives were designed and synthesized. The novel compounds

Figure 1. Chemical structure of 9-hydroxy-5,6-dimethyl-*N*-[2-(dimethylamino)ethyl]-6*H*-pyrido[4,3-*b*]carbazole-1-carboxamide S16020

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Scheme 1. Synthesis of S16020 derivatives

Table 1. Cell growth inhibition of A498 (kidney cancer), A549 (non-small-cell lung cancer) and NHDF (Normal Human Dermal Fibroblasts) cells. IC₅₀ values [μM] ± SD of compounds 8a- 15 compared to ellipticine.

Compound No	A498 IC ₅₀ [μM] ± SD	A549 IC ₅₀ [μM] ± SD	NHDF IC ₅₀ [μM] ± SD
8a	5.03 ± 3.14	1.50 ± 0.29	6.77 ± 0.68
8b	9.17 ± 1.92	10.4 ± 2.15	12.1 ± 1.44
10a	3.24 ± 0.11	2.69 ± 1.16	12.8 ± 0.19
10b	13.3 ± 2.65	19.58 ± 1.57	33.07 ± 7.42
13a	0.78 ± 0.13	0.65 ± 0.3	7.33 ± 1.11
13b	5.42 ± 2.30	4.46 ± 0.44	14.1 ± 1.06
13c	1.30 ± 0.33	7.15 ± 3.83	24.5 ± 4.06
14	2.45 ± 1.03	5.70 ± 4.50	8.6 ± 2.08
15	0.84 ± 0.11	1.25 ± 0.29	15.6 ± 0.71
<i>Ellipticine</i>	1.74 ± 0.04	0.85 ± 0.04	8.93 ± 0.73
<i>Doxorubicin</i>	0.70 ± 0.17	0.99 ± 0.32	6.32 ± 0.55

were synthesized according to Scheme 1. Cytostatic activity *in vitro* of all newly synthesized compounds was examined using kidney cancer (A498), lung cancer (A549) and normal (healthy) human fibroblasts (NHDF) cell lines. Some of the investigated compounds showed strong cytostatic properties with a broad spectrum of antitumor activity when compared to ellipticine. The activity of two of novel compounds is comparable to that exhibited by the reference compound of doxorubicin. All the tested compounds were also characterized by low toxicity to normal cells (Table 1).

Compound **15** (Polish patent- PL-218501) outperformed ellipticine antiproliferative activity on the A498 tumor cell line.

MATERIALS AND METHODS

Instrumentation

Melting points were determined using a Kfler apparatus and were uncorrected; ¹H NMR spectra were recorded on a Bruker 300 at 300. 14 MHz, using TMS as an internal standard. Column chromatography was carried out on silica gel (Merck Kieselgel 100). All of the newly obtained compounds were analyzed for C, H, and N, and the analytical results were within ± 0.4% of theoretical values.

Synthetic chemistry

The starting compound 2-(1-methyl-9*H*-carbazol-2-yl)ethylamine **2** was prepared according to a previously described procedure (24). Compound **2** was reacted with diethyl oxalate and after cycliza-

tion of the resulting amide **3** gave 1-ethoxycarbonyl-5-methyl-3,4-dihydro-6*H*-pyrido[4,3-*b*]carbazole **4**, which was further aromatized to obtain derivative **5**. Compound **6** was obtained via heating of 1-ethoxycarbonyl-5-methyl-6*H*-pyrido[4,3-*b*]carbazole **5** with trifluoroacetic acid (TFA) and urotropine. The 1-ethoxycarbonyl-9-formyl-5-methyl-6*H*-pyrido[4,3-*b*]carbazole **6** was reduced to compound **7**. Derivatives **8a-b** were prepared in reaction of **7** with 2-dimethyl-aminoethylamine or 3-dimethyl-amino-propylamine respectively. Methylation of **5** at N-6 position resulted in derivative **9**. Amides **10a-b** were prepared reacting **9** with 2-dimethyl-aminoethylamine or 2-amino-2-methyl-1-propanol respectively. Compounds **13a-c** were synthesized starting from **9** and using a procedure similar to one described for compounds **8a-b**. Derivative **13a** was boiled in pyridine with 3,3-dimethylglutaric anhydride giving **14**. The most active compound **15** (see Biological Results) was obtained through reaction of **13a** with methyl isocyanate (Scheme 1).

N-[2-(1-methyl-9*H*-carbazole-2-yl)ethyl]-1-ethoxycarbonylamid (**3**)

2-(2-aminoethyl)-1-methylcarbazol **2** (1.12 g, 5 mmol) was dissolved in diethyl oxalate (10 mL). The mixture was heated to 120°C and maintained for 2 h. After evaporation to dryness, the solid was purified using column chromatography and silica gel column, eluting with methylene chloride. Yield: 83%, mp: 183°C. Analysis: calcd. for C₁₉H₂₀N₂O₃: C, 70.35; H, 6.21; N, 8.64%; found: C, 70.10; H, 6.34; N, 8.42%. ¹H NMR (DMSO-*d*₆) δ: 1.25 (m, 3H, CH₂CH₃), 2.52 (s, 3H, 1-CH₃), 2.93 (m, 2H, β-

CH₂), 3.25 (m, 2H, α -CH₂), 4.21 (m, 2H, CH₂CH₃), 6.95 (d, J_{3-4} = 7.9 Hz, 1H, 3-H), 7.10 (d, J_{7-5} = 7.6 Hz, 1H, 7-H), 7.32 (d, J_{6-8} = 8.0 Hz, 1H, 6-H), 7.46 (d, J_{8-6} = 8.0 Hz, 1H, 8-H), 7.83 (d, J_{4-3} = 7.9 Hz, 1H, 4-H), 8.01 (d, J_{5-7} = 7.6 Hz, 1H, 5-H), 9.05 (t, J = 5.2 Hz, 1H, -NHCO), 11.05 (s, 1H, 9-NH).

1-ethoxycarbonyl-5-methyl-3,4-dihydro-6H-pyrido[4,3-b]carbazole (4)

The compound **3** (0.97 g, 3 mmol) was dissolved in boiling toluene (110 mL) and then treated dropwise with phosphorous oxychloride (10 mL). The reflux was continued for 12 h following with evaporation under reduced pressure that resulted in a residue that was solubilized in water (100 mL), alkalized to pH 9-10 with sodium hydrogen carbonate, and extracted with methylene chloride. The extract was dried over magnesium sulfate. After evaporation of the solvent, the solid was purified using column chromatography and silica gel, eluting with methylene chloride: methanol, 98 : 2 v/v. Yield: 43%; mp: 185-186°C. Analysis: calcd. for C₁₉H₁₈N₂O₂: C, 74.49; H, 5.92; N, 9.14%; found: C, 74.34; H, 6.11; N, 8.92%. ¹H NMR (DMSO-d₆) δ : 1.33 (t, 3H, -CH₂CH₃), 2.52 (s, 3H, 5-CH₃), 2.83 (m, 2H, 3-CH₂), 3.72 (m, 2H, 4-CH₂), 4.38 (q, 2H, -CH₂CH₃), 7.17 (t, J_{8-10} = 7.5 Hz, 1H, 8-H), 7.39 (t, J_{9-7} = 8.0 Hz, 1H, 9-H), 7.51 (d, J_{7-9} = 8.0 Hz, 1H, 7-H), 8.02 (s, 1H, 11-H), 8.12 (d, J_{10-8} = 7.7 Hz, 1H, 10-H), 11.50 (s, 1H, 6-NH).

1-ethoxycarbonyl-5-methyl-6H-pyrido[4,3-b]carbazole (5)

A mixture of **4** (0.92 g, 3 mmol), 10% palladium on charcoal (0.10 g) and decalin (50 mL) was stirred at boiling temperature for 30 min. The catalyst was filtered away. The yellow precipitate was filtered and purified using column chromatography and silica gel, eluting with methylene chloride: methanol, 99 : 1 v/v. Yield: 55%; mp: 205°C. Analysis: calcd. for C₁₉H₁₆N₂O₂: C, 74.98; H, 5.30; N, 9.20%; found: C, 74.69; H, 5.45; N, 9.01%. ¹H NMR (DMSO-d₆) δ : 1.42 (t, 3H, -CH₂CH₃), 2.87 (s, 3H, 5-CH₃), 4.55 (q, 2H, -CH₂CH₃), 7.26 (m, 1H, 8-H), 7.55 (m, 2H, 7-H, 9-H), 8.15 (d, J_{4-3} = 6.0 Hz, 1H, 4-H), 8.34 (d, J_{10-8} = 7.7 Hz, 1H, 10-H), 8.45 (d, J_{3-4} = 5.9 Hz, 1H, 3-H), 8.97 (s, 1H, 11-H), 11.55 (s, 1H, 6-NH).

1-ethoxycarbonyl-9-formyl-5-methyl-6H-pyrido[4,3-b]carbazole (6)

1-ethoxycarbonyl-9-formyl-5,6-dimethyl-6H-pyrido[4,3-b]carbazole (11)

A mixture of 0.5 mmol compound **5** (or compound **9**), 20 mL trifluoroacetic acid and 0.71 g (0.5

mmol) hexamethylenetetramine was stirred at boiling temperature for 20 min. After evaporation under reduced pressure the resulting compound was solubilized in water (100 mL), alkalized to pH 9-10 with sodium hydrogen carbonate, and extracted with methylene chloride. The extract was dried over magnesium sulfate. After evaporation of the solvent, the solid was purified using column chromatography and silica gel, eluting with methylene chloride: methanol, 99 : 1 v/v.

1-ethoxycarbonyl-9-formyl-5-methyl-6H-pyrido[4,3-b]carbazole (6)

Yield: 36%; mp: 194-195°C. Analysis: calcd. for C₂₀H₁₆N₂O₃: C, 72.28; H, 4.85; N, 8.43%; found: C, 72.10; H, 5.04; N, 8.20%. ¹H NMR (DMSO-d₆) δ : 1.42 (t, 3H, -CH₂CH₃), 2.87 (s, 3H, 5-CH₃), 4.57 (q, 2H, -CH₂CH₃), 7.66 (d, J_{8-7} = 8.3, 1H, 8-H), 8.07 (d, J_{7-8} = 8.4 Hz, 1H, 7-H), 8.18 (d, J_{4-3} = 6.0 Hz, 1H, 4-H), 8.48 (d, J_{3-4} = 6.0 Hz, 1H, 3-H), 8.93 (s, 1H, 10-H), 9.11 (s, 1H, 11-H), 10.07 (s, 1H, -CHO), 12.08 (s, 1H, 6-NH).

1-ethoxycarbonyl-9-formyl-5,6-dimethyl-6H-pyrido[4,3-b]carbazole (11)

Yield: 81%; mp: 240°C. Analysis: calcd. for C₂₁H₁₈N₂O₃: C, 72.82; H, 5.24; N, 8.09%; found: C, 72.58; H, 5.37; N, 7.82%. ¹H NMR (DMSO-d₆) δ : 1.42 (t, 3H, -CH₂CH₃), 3.11 (s, 3H, 5-CH₃), 4.24 (s, 3H, 6-CH₃), 4.56 (q, 2H, -CH₂CH₃), 7.80 (d, J_{8-7} = 8.4, 1H, 8-H), 8.11 (d, J_{7-8} = 8.4 Hz, 1H, 7-H), 8.26 (d, J_{4-3} = 6.1 Hz, 1H, 4-H), 8.50 (d, J_{3-4} = 6.1 Hz, 1H, 3-H), 8.93 (s, 1H, 10-H), 9.11 (s, 1H, 11-H), 10.08 (s, 1H, -CHO).

1-ethoxycarbonyl-9-hydroxymethyl-5-methyl-6H-pyrido[4,3-b]carbazole (7)

1-ethoxycarbonyl-9-hydroxymethyl-5,6-dimethyl-6H-pyrido[4,3-b]carbazole (12)

The compound **6** 0.5 mmol or compound **12** 0.5 mmol, 90 mL THF and 0.20 g (5 mmol) sodium borohydride were stirred at room temperature for 2 h. After evaporation under reduced pressure the resulting compound was solubilized in water (100 mL), acidified to pH 4-5 with hydrochloric acid, alkalized to pH 9-10 with sodium hydrogen carbonate, and extracted with methylene chloride. The extract was dried over magnesium sulfate. After evaporation of the solvent, the solid was purified using column chromatography and silica gel, eluting with methylene chloride: methanol, 98 : 2 v/v.

1-ethoxycarbonyl-9-hydroxymethyl-5-methyl-6H-pyrido[4,3-b]carbazole (7)

Yield: 66%; mp: 243°C. Analysis: calcd. for $C_{20}H_{18}N_2O_3$: C, 71.84; H, 5.43; N, 8.38%; found: C, 71.66; H, 5.63; N, 8.11%. 1H NMR (DMSO- d_6) δ : 1.43 (t, 3H, $-CH_2CH_3$), 2.87 (s, 3H, 5- CH_3), 4.56 (q, 2H, $-CH_2CH_3$), 4.67 (s, 2H, $-CH_2OH$), 5.27 (s, 1H, -OH), 7.53 (m, 2H, 8-H, 7-H), 8.15 (d, $J_{4-3} = 6.0$ Hz, 1H, 4-H), 8.26 (s, 1H, 10-H), 8.45 (d, $J_{3-4} = 6.0$ Hz, 1H, 3-H), 8.96 (s, 1H, 11-H), 11.60 (s, 1H, 6-NH).

1-ethoxycarbonyl-9-hydroxymethyl-5,6-dimethyl-6H-pyrido[4, 3-b]carbazole (12)

Yield: 58%; mp: 196-197°C. Analysis: calcd. for $C_{21}H_{20}N_2O_3$: C, 72.40; H, 5.79; N, 8.04%; found: C, 72.27; H, 5.98; N, 7.83%. 1H NMR (DMSO- d_6) δ : 1.39 (t, 3H, $-CH_2CH_3$), 3.08 (s, 3H, 5- CH_3), 4.15 (s, 3H, 6- CH_3), 4.55 (q, 2H, $-CH_2CH_3$), 4.67 (s, 2H, $-CH_2OH$), 5.27 (s, 1H, -OH), 7.56 (m, 2H, 8-H, 7-H), 8.20 (d, $J_{4-3} = 6.0$ Hz, 1H, 4-H), 8.26 (s, 1H, 10-H), 8.44 (d, $J_{3-4} = 6.0$ Hz, 1H, 3-H), 8.95 (s, 1H, 11-H).

The synthesis of amides 8a-b, 10a-b, 13a-c

A mixture of (0.5 mmol) 6H-pyrido[4,3-b]carbazole derivative **7** (or **9** or **12**) and 10 mL corresponding amine was heated at reflux under nitrogen atmosphere. The reaction was performed until the disappearance of the starting material (TLC monitoring). After evaporation of the solvent, the solid was purified using column chromatography and silica gel, eluting with methylene chloride: methanol, 95 : 5 v/v.

9-hydroxymethyl-5-methyl-1-[N,N-(dimethylamino)ethyl]carbamoyl-6H-pyrido[4,3-b]carbazole (8a)

Yield: 57%; mp: 248-249°C. Analysis: calcd. for: $C_{22}H_{24}N_4O_2$: C, 70.19; H, 6.43; N, 14.88%; found: C, 69.99; H, 6.56; N, 14.73%. 1H NMR (DMSO- d_6) δ : 2.24 (s, 6H, $-N(CH_3)_2$), 2.52 (m, 2H, β - CH_2), 2.84 (s, 3H, 5- CH_3), 3.50 (q, 2H, α - CH_2), 4.66 (s, 2H, $-CH_2OH$), 5.23 (s, 1H, -OH), 7.49 (m, 2H, 8-H, 7-H), 8.11 (d, $J_{4-3} = 6.0$ Hz, 1H, 4-H), 8.18 (s, 1H, 10-H), 8.42 (d, $J_{3-4} = 6.0$ Hz, 1H, 3-H), 8.90 (t, 1H, $-CONHCH_2$), 9.66 (s, 1H, 11-H), 11.41 (s, 1H, 6-NH).

9-hydroxymethyl-5-methyl-1-[N,N-(dimethylamino)propyl]carbamoyl-6H-pyrido[4,3-b]carbazole (8b)

Yield: 51%; mp: 244°C. Analysis: calcd. for $C_{23}H_{26}N_4O_2$: C, 70.75; H, 6.71; N, 14.35%; found: C, 70.57; H, 6.92; N, 14.13%. 1H NMR (DMSO- d_6) δ : 1.74 (m, 2H, β - CH_2), 2.15 (s, 6H, $-N(CH_3)_2$), 2.32 (t, 2H, γ - CH_2), 2.82 (s, 3H, 5- CH_3), 3.43 (m, 2H, α - CH_2), 4.66 (s, 2H, $-CH_2OH$), 5.26 (s, 1H, -OH), 7.50

(m, 2H, 8-H, 7-H), 8.09 (d, $J_{4-3} = 6.0$ Hz, 1H, 4-H), 8.17 (s, 1H, 10-H), 8.41 (d, $J_{3-4} = 6.0$ Hz, 1H, 3-H), 8.90 (t, $J_{NH-CH_2} = 5.8$ Hz, 1H, $-CONHCH_2$), 9.50 (s, 1H, 11-H), 11.59 (s, 1H, 6-NH).

5,6-dimethyl-1-[N,N-(dimethylamino)ethyl]carbamoyl-6H-pyrido[4,3-b]carbazole (10a)

Yield: 44%; mp: 171-172°C. Analysis: calcd. for $C_{22}H_{24}N_4O$: C, 73.31; H, 6.71; N, 15.54%; found: C, 73.07; H, 6.84; N, 15.29%. 1H NMR (DMSO- d_6) δ : 2.24 (s, 6H, $-N(CH_3)_2$), 2.52 (m, 2H, β - CH_2), 3.05 (s, 3H, 5- CH_3), 3.49 (m, 2H, α - CH_2), 4.12 (s, 3H, 6- CH_3), 7.27 (m, 1H, 8-H), 7.58 (m, 2H, 9-H, 7-H), 8.15 (d, $J_{4-3} = 6.1$ Hz, 1H, 4-H), 8.20 (d, $J_{10-8} = 7.6$ Hz, 1H, 10-H), 8.41 (d, $J_{3-4} = 6.1$ Hz, 1H, 3-H), 8.76 (t, 1H, $-CONHCH_2$), 9.66 (s, 1H, 11-H).

5,6-dimethyl-1-(2-hydroxy-1,1-dimethylethyl)carbamoyl-6H-pyrido[4,3-b]carbazole (10b)

Yield: 34%; mp: 213°C. Analysis: calcd. for $C_{22}H_{23}N_3O_2$: C, 73.11; H, 6.41; N, 11.63%; found: C, 72.92; H, 6.56; N, 11.43%. 1H NMR (DMSO- d_6) δ : 1.50 (s, 6H, $-(CH_3)_2$), 3.06 (s, 3H, 5- CH_3), 3.59 (s, 2H, $-CH_2OH$), 4.13 (s, 3H, 6- CH_3), 5.16 (s, 1H, OH), 7.27 (m, 1H, 8-H), 7.57 (m, 2H, 9-H, 7-H), 8.15 (d, $J_{4-3} = 6.1$ Hz, 1H, 4-H), 8.21 (d, $J_{10-8} = 7.5$ Hz, 1H, 10-H), 8.39 (d, $J_{3-4} = 6.0$ Hz, 1H, 3-H), 8.43 (s, 1H, $-CONH$), 9.70 (s, 1H, 11-H).

9-hydroxymethyl-5,6-dimethyl-1-[N,N-(dimethylamino)ethyl]carbamoyl-6H-pyrido[4,3-b]carbazole (13a)

Yield: 44%; mp: 131-132°C. Analysis: calcd. for $C_{23}H_{26}N_4O_2$: C, 70.75; H, 6.71; N, 14.35%; found: C, 70.52; H, 6.82; N, 14.09%. 1H NMR (DMSO- d_6) δ : 2.11 (m, 2H, β - CH_2), 2.24 (s, 6H, $-N(CH_3)_2$), 3.11 (s, 3H, 5- CH_3), 3.50 (q, 2H, α - CH_2), 4.13 (s, 3H, 6- CH_3), 4.67 (s, 2H, $-CH_2OH$), 5.28 (s, 1H, -OH), 7.53 (m, 2H, 8-H, 7-H), 8.16 (m, 2H, 4-H, 10-H), 8.42 (d, $J_{3-4} = 6.0$ Hz, 1H, 3-H), 8.77 (t, 1H, $-CONHCH_2$), 9.66 (s, 1H, 11-H).

9-hydroxymethyl-5,6-dimethyl-1-[N,N-(dimethylamino)propyl]carbamoyl-6H-pyrido[4,3-b]carbazole (13b)

Yield: 33%; mp: 129-130°C. Analysis: calcd. for $C_{24}H_{28}N_4O_2$: C, 71.26; H, 6.98; N, 13.85%; found: C, 71.11; H, 7.16; N, 13.58%. 1H NMR (DMSO- d_6) δ : 1.74 (m, 2H, β - CH_2), 2.19 (s, 6H, $-N(CH_3)_2$), 2.37 (t, 2H, γ - CH_2), 3.07 (s, 3H, 5- CH_3), 3.39 (m, 2H, α - CH_2), 4.15 (s, 3H, 6- CH_3), 4.67 (s, 2H, $-CH_2OH$), 5.25 (s, 1H, -OH), 7.54 (m, 2H, 8-H, 7-H), 8.16 (m, 2H, 4-H, 10-H), 8.42 (d, $J_{3-4} = 6.0$ Hz, 1H, 3-H), 8.93 (t, $J_{NH-CH_2} = 5.7$ Hz, 1H, $-CONHCH_2$), 9.54 (s, 1H, 11-H).

9-hydroxymethyl-5,6-dimethyl-1-(2-hydroxy-1,1-dimethylethyl)carbamoyl-6H-pyrido[4,3-b]carbazole (13c)

Yield: 37%; mp: 205–206°C. Analysis: calcd. for $C_{23}H_{25}N_3O_3$: C, 70.57; H, 6.44; N, 10.73%; found: C, 70.35; H, 6.63; N, 10.58%. 1H NMR (DMSO- d_6) δ : 1.44 (s, 6H, $-(CH_3)_2$), 3.06 (s, 3H, 5- CH_3), 3.57 (s, 2H, $-CH_2OH$), 4.13 (s, 3H, 6- CH_3), 4.67 (s, 2H, 9- CH_2OH), 5.16 (s, 1H, $-CH_2OH$), 5.27 (s, 1H, 9- CH_2OH), 7.54 (m, 2H, 8-H, 7-H), 8.16 (m, 2H, 4-H, 10-H), 8.39 (d, $J_{3,4} = 6.0$ Hz, 1H, 3-H), 8.44 (s, 1H, $-CONH$), 9.73 (s, 1H, 11-H).

1-ethoxycarbonyl-5,6-dimethyl-6H-pyrido[4,3-b]carbazole (9)

A mixture of 1-ethoxycarbonyl-5-methyl-6H-pyrido[4,3-b]carbazole **5** (0.60 g, 2 mmol), 1.0 g dry potassium carbonate, 30 mL dimethyl carbonate, 3 mL DMF and 0.02 g 18-crown-6 was stirred at boiling temperature for 24 h. After evaporation under reduced pressure the resulting compound was solubilized in water (100 mL) and extracted with methylene chloride. The extract was dried over magnesium

sulfate. After evaporation of the solvent, the solid was purified using column chromatography and silica gel, eluting with methylene chloride: methanol, 99:1 v/v. Yield: 88% mp: 191°C. Analysis: calcd. for $C_{20}H_{18}N_2O_2$: C, 75.45; H, 5.70; N, 8.80%; found: C, 75.24; H, 5.89; N, 8.71%. 1H NMR (DMSO- d_6) δ : 1.42 (t, 3H, $-CH_2CH_3$), 3.10 (s, 3H, 5- CH_3), 4.10 (s, 3H, 6- CH_3), 4.56 (q, 2H, $-CH_2CH_3$), 7.29 (m, 1H, 8-H), 7.61 (m, 2H, 7-H, 9-H), 8.22 (d, $J_{4,3} = 6.3$ Hz, 1H, 4-H), 8.33 (d, $J_{10,8} = 7.6$ Hz, 1H, 10-H), 8.45 (d, $J_{3,4} = 6.3$ Hz, 1H, 3-H), 8.96 (s, 1H, 11-H).

9-(3-carboxy-2,2-dimethylpropyl)acetate-5,6-dimethyl-1-[N,N-(dimethylamino)ethyl]carbamoyl-6H-pyrido[4,3-b]carbazole (14)

3,3-Dimethylglutaric anhydride 0.14 g (1 mmol) was added to a mixture of 5,6-dimethyl-9-hydroxymethyl-1-[N,N-(dimethylamino)ethyl]carbamoyl-6H-pyrido[4,3-b]carbazole **13a** in 20 mL of pyridine, anhydrous. The mixture was stirred at boiling temperature for 24 h. After evaporation of the solvent, the solid was purified using column chromatography and silica gel, eluting with methylene chloride: methanol, 9 : 1 v/v. Yield: 28% mp: 107–108°C. Analysis: calcd. for $C_{30}H_{36}N_4O_5$: C, 67.65; H, 6.81; N, 10.52%; found: C, 67.42; H, 6.92; N, 10.33%. 1H NMR (DMSO- d_6) δ : 1.03 (s, 6H, $-(CH_3)_2$), 2.27 (s, 2H, $-CH_2COOCH_2$), 2.32 (s, 6H, $-N(CH_3)_2$), 2.44 (s, 2H, $HOOCCH_2$), 2.77 (m, 2H, β - CH_2), 3.08 (s, 3H, 5- CH_3), 3.49 (m, 2H, α - CH_2), 4.16 (s, 3H, 6- CH_3), 5.25 (s, 2H, 9- CH_2), 7.61 (m, 2H, 8-H, 7-H), 8.19 (d, 2H, $J_{4,3} = 6.1$ Hz, 1H, 4-H), 8.25 (s, 1H, 10-H), 8.44 (d, $J_{3,4} = 6.0$ Hz, 1H, 3-H), 8.44 (s, 1H, $-CONH$), 9.65 (s, 1H, 11-H).

5,6-dimethyl-1-[N,N-(dimethylamino)ethyl]carbamoyl-9-(N-methylcarbamoyloxymethyl)-6H-pyrido[4,3-b]carbazole (15)

A mixture of compound **13a** 0.38 g (1 mmol), 50 mL dry chloroform and 0.36 g (3 mmol) 4-dimethylaminopyridine (DMAP) was stirred at room temperature for 0.5 h, following with addition of 5 mL methyl isocyanate at room temperature over 2 h. The resulting mixture was solubilized in water (100 mL) and extracted with chloroform. The extract was dried over magnesium sulfate. After evaporation of the solvent, the solid was purified using column chromatography and silica gel, eluting with chloroform: methanol, 99 : 1 v/v.

Yield: 60% mp: 185–186°C. Analysis: calcd. for $C_{25}H_{29}N_5O_3$: C, 67.13; H, 6.48; N, 15.65%; found: C, 67.05; H, 6.53; N, 15.73%. 1H NMR (DMSO- d_6) δ = ppm: 2.25 (s, 6H, 2 x CH_3), 2.53 (m, 2H, β - CH_2), 2.59 (d, $J = 4.5$ Hz, 3H, CH_3NH), 3.09

Figure 2. Cytotoxicity evaluation of compound **15** (10 μ M) for A549 (A) and NHDF (B) cells. Dyeing with propidium iodide (red cells – dead). Magnification of the 10 x lens, Ex: 531 nm Em: 593 nm

(s, 3H, 5-CH₃), 3.51 (m, 2H, α -CH₂), 4.12 (s, 3H, 6-CH₃), 5.18 (s, 2H, 9-CH₂), 7.11 (d, $J = 4.4$ Hz, 1H, CH₃NH), 7.65 (s, 2H, 8-H+ 7-H), 8.15 (d, $J_{4-3} = 6.1$ Hz, 1H, 4-H), 8.19 (s, 1H, 10-H), 8.42 (d, $J_{3-4} = 6.1$ Hz, 1H, 3-H), 8.78 (t, $J = 5.5$ Hz, 1H, CONH-CH₂), 9.66 (s, 1H, 11-H).

Biological tests

Test solutions of nine new pyrido[4,3-*b*]carbazole derivatives **8 a-b**, **10 a-b**, **13 a-c**, **14**, **15** and ellipticine (1 mg/mL) were prepared *ex tempore* for each test by dissolving each of the compounds in 100 μ L of DMSO and 900 μ L of culture media. The solutions were diluted in cell culture media to reach the final concentrations of 100 to 0.0001 μ g/mL. The compounds were weighed (Sartorius) with an accuracy of 0.00001 g.

Cell lines

The human cancer cell lines A498 (kidney cancer) and A549 (non-small-cell lung cancer) were used. The lines were cultured by the Cell Culture Collection of the Department of Tumor Immunology, Institute of Immunology and Experimental Therapy, Wrocław, Poland. The normal cells (normal human dermal fibroblasts – NHDF) were purchased from LONZA (Verviers, Belgium).

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 μ g/mL). The A498 cells were cultivated in Eagle medium supplemented with 10% serum (FCS), sodium pyruvate (1 mM), glutamine (2 mM), penicillin (100 U/mL), and streptomycin (100 μ g/mL). The NHDF cells were cultivated in DMEM medium supplemented with 10% fetal bovine serum (FBS), glutamine (2 mM), penicillin (100 U/mL), and streptomycin (100 μ g/mL). The cell cultures were maintained at 37°C in a humid atmosphere containing 5% CO₂.

SRB

The SRB method was used as described by Skehan et al. (21). 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 effect of DMSO on the proliferation of selected cell lines was tested.

DISCUSSION

The nine newly synthesized compounds were subjected to the preliminary *in vitro* tests for their cytostatic activity against two human tumor cell lines

A498 (kidney cancer) and A549 (lung cancer) in comparison to reference compounds, ellipticine and doxorubicin (standard cytostatic). Pharmacological results were outlined in Table 1. All compounds exhibited biological activity. Compounds with substituents at 1 and 9 positions (**13a**, **13b**, **13c**, **14**, **15**) showed significant antiproliferative properties. The results of the *in vitro* biological study indicated that the presence of [*N,N*-(dimethylamino)ethyl]carbamoyl moieties at 1 position of 6*H*-pyrido[4,3-*b*]carbazole ring system significantly increases the cytostatic properties of synthesized compounds when compared to [*N,N*-(dimethylamino)propyl]carbamoyl moieties at 1 position of 6*H*-pyrido[4,3-*b*]carbazole. Compound **13a** outperformed the antiproliferative action on both tumor cell lines tested when compared to ellipticine and doxorubicin. New pyridocarbazole derivative **15** (Polish patent PL-218501) showed significant antiproliferative properties comparable to reference ellipticine and doxorubicin on human tumor cell lines A498. The compounds **15** and **8a** showed biological activity only slightly worse or comparable to reference ellipticine and doxorubicin on human tumor cell lines A549. All the tested compounds also showed lower toxicity towards normal cells (NHDF). High antiproliferative activity of the new derivatives in relation to cancer cells and low toxicity to normal cells are an important feature of the synthesized compounds (Fig. 2).

As it has been shown previously (3), application of structure-activity relationship (SAR) to design of new active compounds can result in the determination of the chemical group responsible for induction of a desired biological effect. In our case, SAR based approach produced derivatives that outperformed the reference compound in several tests. Further investigation of the reported compounds is in progress.

Conflict of interest

The authors declare that there is no conflict of interest.

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