

The synthesis and biological properties of a 1-(2-methylpyridin-4-yl) olivacine derivative

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Abstract

Starting from 2-(6-methoxy-1-methyl-9H-carbazol-2-yl)ethylamine and 2-methylisonicotinic acid, 9-hydroxy-5,6-dimethyl-1-(2-methylpyridin-4-yl)-6H-pyrido[4,3-*b*]carbazole (**5**) was obtained. The new compound showed significant cytostatic activity for cultured L1210 cells and no inhibition of growth of the *E. coli* O56 strain was observed. The bactericidal activity of normal human serum against *E. coli* O56 was not affected by the examined compound **5** and its isomer **4**.

Keywords

Olivacine, 1-substituted-6H-pyrido[4,3-*b*]carbazole, cytotoxicity

Introduction

The high *in vitro* anticancer activity of the natural alkaloids ellipticine **1** and its isomer olivacine **1a** (Fig.1) provokes great interest in modifying their structure to obtain new derivatives with a more advantageous therapeutic index.

Recently, our further modifications of the structure of olivacine have led to new derivatives, such as **2 – 4**, which are presented in Fig. 2 and which have shown significant cytostatic activity [1-3].

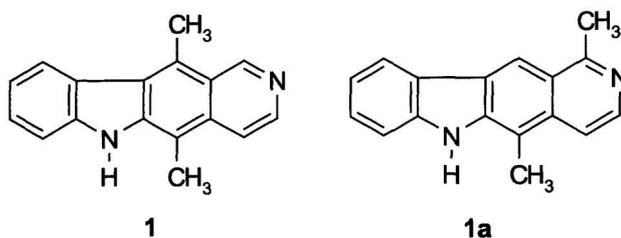


Fig. 1. The structure of ellipticine (1) and its isomer olivacine (1a)

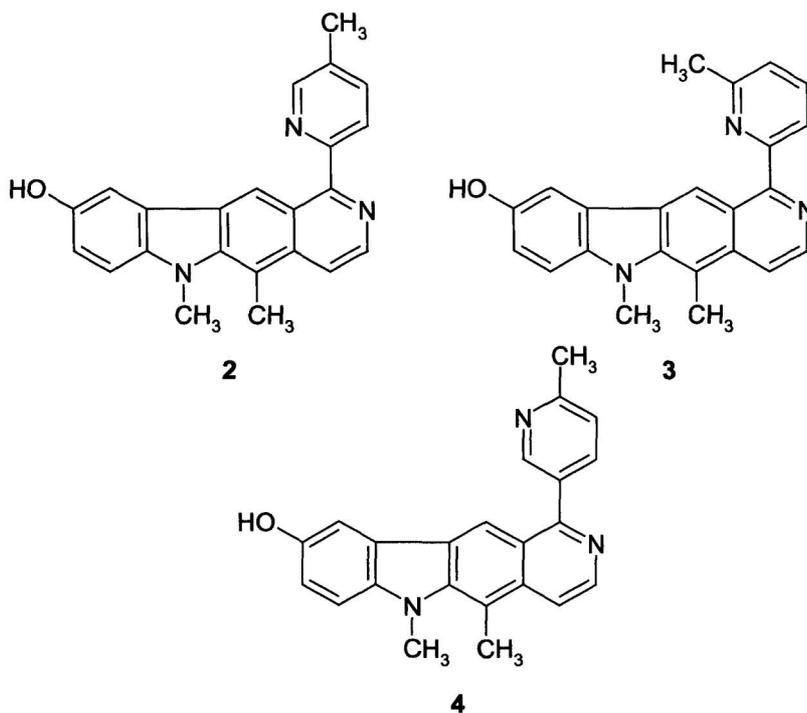


Fig. 2. Recent modifications of the structure of olivacine

The high antitumor (*in vitro*) activity of the olivacine derivatives mentioned above prompted us to prepare the new 1-(pyridin-4-yl)-substituted olivacine

derivative **5**, to examine its cytostatic activity and to determine and compare the influence of olivacine derivatives **4** and **5** on the growth of the *Escherichia coli* O56 strain and the level of bactericidal activity of NBS (normal bovine serum) against *E. coli* O56.

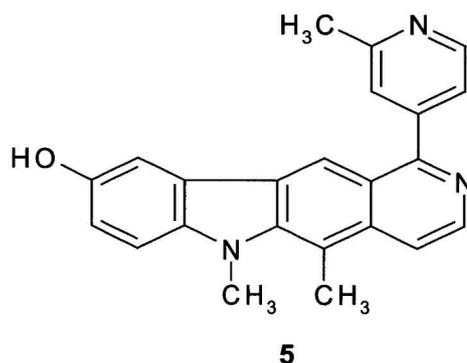
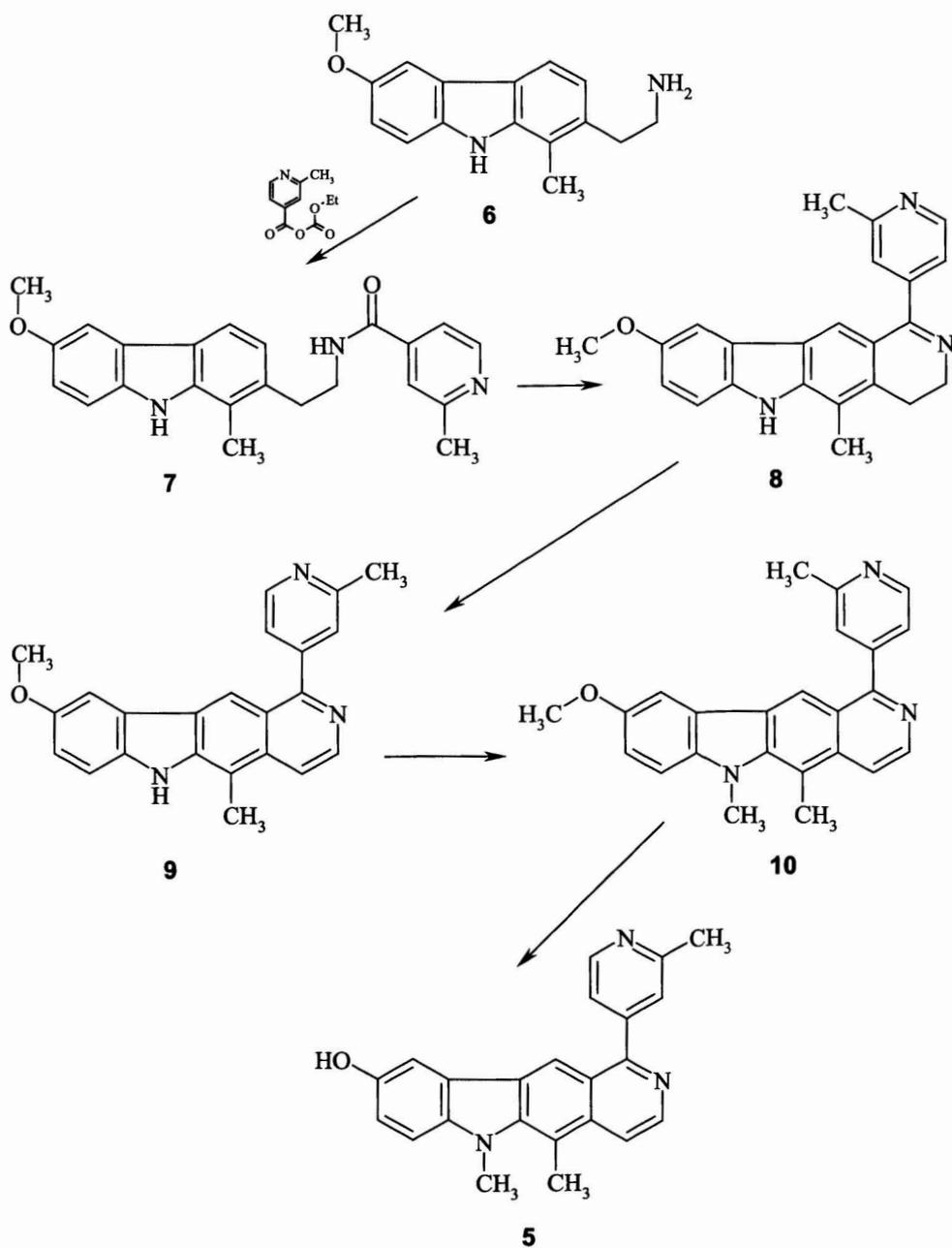


Fig.3. Structure of the newly obtained pyrido[4,3-b]carbazole derivative **5**

The bactericidal activity of serum caused by the complement system is an important defence mechanism protecting the host organism against infections caused by Gram-negative bacteria [4]. Complement plays the most important role in eliminating bacterial invasion of the host by facilitating phagocytosis of potential pathogens and participating in the direct killing of susceptible Gram-negative bacteria [5].

This pyrido[4,3-b]carbazole derivative **5** was obtained according to Scheme 1 below.



Scheme 1

Results and Discussion

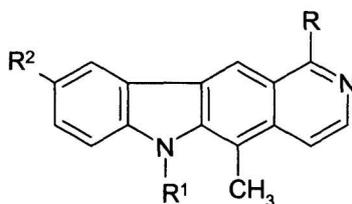
The starting compound, 2-(6-methoxy-1-methyl-9H-carbazol-2-yl)ethylamine (**6**), has already been described [6]. It was allowed to react with a mixed anhydride of 2-methylisonicotinic acid and cyclization of the resulting amide **7** with phosphorus oxychloride in boiling toluene gave 9-methoxy-5-methyl-1-(2-methylpyridin-4-yl)-3,4-dihydro-6H-pyrido[4,3-b]carbazole (**8**), which was aromatized to derivative **9** by dehydrogenation over 10% palladium on charcoal in boiling diphenyl ether.

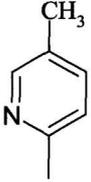
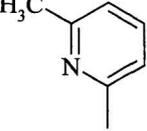
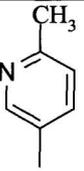
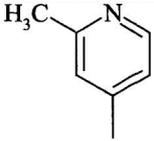
N-6-Methylation of **9** to **10** was performed by using an excess of dimethyl carbonate in dimethylformamide in the presence of potassium carbonate and 18-Crown-6. This last compound **10** was 9-O-demethylated to 5,6-dimethyl-9-hydroxy-1-(2-methylpyridin-4-yl)-6H-pyrido[4,3-b]carbazole (**5**) by heating with 48% aqueous hydrobromic acid.

The biological properties of the new pyrido[4,3-b]carbazole derivatives (**5**) were first evaluated *in vitro* on a L1210 leukemia cell line (ATCC CCL219) according to procedures already described [2].

Cytostatic activity was expressed as IC₅₀, the concentration that reduced the growth of treated cells by 50% relative to controls. Results were measured from regression curves obtained with experimental points free of significant toxicity and are summarized in Table 1 in comparison with olivacine.

Tab. 1. Cytostatic properties (IC₅₀ values, μM) of compounds **2-5** in comparison with olivacine



Compounds No	R ¹	R ²	R	L 1210 IC ₅₀ [μM]
Olivacine	H	H	CH ₃	2.03 ^[7]
2	CH ₃	OH		1.7 ^[1]
3	CH ₃	OH		0.9 ^[2]
4	CH ₃	OH		0.85 ^[3]
5	CH ₃	OH		0.8

As shown in Table 1, all products were more active than olivacine, depending on the kind of substituent at position 1 in the main heterocyclic system, the analogues (**4** and **5**) of the predecessor **2** display *in vitro* cytostatic activity of the same order.

The complement system plays a key role in the defence against microorganisms, and the bactericidal activity of serum depends on many different factors. The O-specific side chains of lipopolysaccharides (LPS) and outer membrane proteins (OMPs) play a decisive role in this phenomenon [4,5,8]. The aim of this study was to determine and compare the influence of olivacine

derivatives **4** and **5** on the growth of bacteria *E. coli* O56 and compare the level of bactericidal activity of NBS against the *E. coli* O56 strain.

Tab. 2. Growth of the *E. coli* O56 strain in YP in the presence of olivacine derivatives **4** and **5**

Time of incubation /min/	Control		Olivacine derivative 4		Control		Olivacine derivative 5	
	c.f.u.*	Percent survival	c.f.u.	Percent survival	c.f.u.	Percent survival	c.f.u.	Percent survival
0	6.5x10 ⁶	100	6.3x10 ⁶	100	5.0x10 ⁶	100	4.9x10 ⁶	100
60	31.6x10 ⁶	471	43.2x10 ⁶	685	19.8x10 ⁶	391	128x10 ⁶	258
180	62.3x10 ⁶	925	77.2x10 ⁶	1222	22.8x10 ⁶	570	343x10 ⁶	691

* c.f.u., colony-forming units

The results presented in Table 2 show no inhibitory effect of olivacine derivatives **4** and **5** on the multiplication of *E. coli* O56 strain. The number of bacterial cells growing in the presence or absence of the examined compounds was nearly equal. A similar situation was observed in the investigation of anticomplement activity of derivatives **4** and **5**.

The results shown in Table 3 give no indication that olivacine derivatives **4** and **5** influence the level of activation and cytolytic effect of complement of normal bovine serum.

Tab. 3. Effect of olivacine derivatives on the bactericidal activity of 12.5% normal bovine serum against *E. coli* O56

Time of incubation (min)	Control		Olivacine derivative 4		Control		Olivacine derivative 5	
	c.f.u.*	Percent survival	c.f.u.	Percent survival	c.f.u.	Percent survival	c.f.u.	Percent survival
0	2.3x10 ⁶	100	1.5x10 ⁶	100	4.2x10 ⁶	100	2.9x10 ⁶	100
60	6.7x10 ³	0.29	4.3x10 ³	0.28	2.4x10 ⁴	0.57	2.0x10 ⁴	0.68
180	5.0x10 ³	0.21	3.6x10 ³	0.24	1.7x10 ⁴	0.40	1.7x10 ⁴	0.58

* c.f.u., colony-forming units

These data indicate the possibility of using these olivacine derivatives as cytostatic drugs in the therapy of tumor diseases. The results also indicate that the olivacine derivatives show higher cytostatic activity than olivacine.

Experimental

Melting points were determined on a Kofler apparatus and were uncorrected; ^1H NMR spectra were recorded on a Tesla BS 587 A at 80 MHz or on a Bruker 300 at 300.14 MHz, using TMS as the 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 $\pm 0.4\%$ of the theoretical values.

2-(6-methoxy-1-methylcarbazol-2-yl)ethylamine (6) see[6].

5,6-Dimethyl-9-hydroxy-1-(6-methylpyridin-3-yl)-6H-pyrido[4,3-b]carbazole (4) see [3].

***N*-[2-(6-Methoxy-1-methyl-9H-carbazol-2-yl)ethyl]-2-methyl-4-pyridinecarboxamide (7)**

Triethylamine (0.405 g, 4 mmol) was added to 2-methyl-4-pyridinecarboxylic acid (452 mg, 3.3 mmol) in dry methylene chloride (100 ml). After cooling to -10°C , a solution of ethyl chloroformate (0.435 g, 4 mmol) in dry methylene chloride (10 ml) was added to the resulting mixture under stirring. The mixture was stirred for a further 30 min and then 2-(6-methoxy-1-methylcarbazol-2-yl)ethylamine **6** (763 mg, 3 mmol) in THF (100 ml) was added drop-wise at -10°C . The resulting mixture was allowed to reach room temperature (20 h) under stirring and then the precipitate was collected and the filtrate was evaporated to dryness. The residue was taken up in water (50 ml), basified with concentrated aqueous ammonia and extracted with methylene chloride, then dried over magnesium sulfate. Evaporation of the solvent provided a solid residue, which was recrystallized from ethanol to give 228 mg

(20.5%) of amide, mp 195-6°C. Anal. Calc'd for $C_{23}H_{23}N_3O_2$: C, 73.97; H, 6.21; N, 11.25. Found: C, 73.56; H, 6.54; N, 10.91. 1H NMR (DMSO- d_6) δ : 2.58 (s, 3H, 2'-CH₃), 2.68 (s, 3H, 1-CH₃), 2.75 (t, $J_{\alpha-CH_2-\beta-CH_2} = 6.7$ Hz, 2H, α -CH₂), 3.31 (t, 2H, β -CH₂), 3.88 (s, 3H, 6-OCH₃), 7.04 (dd, $J_{8-7} = 8.8$ Hz, $J_{7-5} = 2.3$ Hz, 1H, 7-H), 7.39 (m, 2H, 3-H + 3'-H), 7.71 (d, $J_{8-7} = 8.7$ Hz, 1H, 8-H), 7.83 (d, $J_{5-7} = 2.3$ Hz, 1H, 5-H), 7.99 (dd, $J_{5-6'} = 5.4$ Hz, $J_{5'-3'} = 1.8$ Hz, 1H, 5'-H), 8.65 (d, $J_{4-3} = 8.0$ Hz, 1H, 4-H), 9.00 (d, $J_{6'-5} = 5.3$ Hz, 1H, 6'-H), 10.42 (s, 1H, 9-H).

9-Methoxy-5-methyl-1-(2-methylpyridin-4-yl)-3,4-dihydro-6H-pyrido[4,3-b]carbazole (8)

The amide **7** (747 mg, 2 mmol) was dissolved in boiling toluene (70 ml) and treated drop-wise with phosphorous oxychloride (5 ml). Reflux was continued for a 12 h period, and evaporation under reduced pressure afforded a residue which was taken up in water (100ml), basified to pH 9-10 with concentrated aqueous ammonia, and extracted with methylene chloride. Evaporation of the solvent provided a solid residue, which was recrystallized from ethanol to give yellow crystals (320 mg, 45%), mp 269-70°C. Anal. Calc'd for $C_{23}H_{21}N_3O$: C, 77.72; H, 5.96; N, 11.82. Found: C, 77.52; H, 6.03; N, 11.91. 1H NMR (CDCl₃) δ : 2.50 (s, 3H, 2'-CH₃), 2.63 (s, 3H, 5-CH₃), 2.97 (m, 2H, 4-H), 3.70 (m, 2H, 3-H), 3.91 (s, 3H, 9-OCH₃), 7.01 (dd, $J_{8-7} = 8.6$ Hz, $J_{8-10} = 2.3$ Hz, 1H, 8-H), 7.26 (m, 1H, 3'-H), 7.38 (d, $J_{7-8} = 8.9$ Hz, 1H, 7-H), 7.69 (m, 2H, 5'-H + 10-H), 7.89 (s, 1H, 11-H), 8.86 (d, $J_{5'-6'} = 4.9$ Hz, 1H, 6'-H), 9.76 (s, 1H, 6-H).

9-Methoxy-5-methyl-1-(2-methylpyridin-4-yl)-6H-pyrido[4,3-b]carbazole (9)

Compound **8** (711 mg, 2 mmol) was refluxed in diphenyl ether (25 ml) in the presence of 10% palladized charcoal (80 mg) for 30 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 from ethyl acetate to give yellow crystals (700 mg, 99%), mp 309°C. Anal. Calc'd for $C_{23}H_{19}N_3O$: C, 78.16; H, 5.42; N, 11.89. Found: C, 77.97; H, 5.66; N, 11.73. 1H NMR (CDCl₃) δ : 2.75 (s, 3H,

2'-CH₃), 2.87 (s, 3H, 5-CH₃), 3.93 (s, 3H, 9-OCH₃), 7.09 (dd, J₈₋₇ = 8.8 Hz, J₈₋₁₀ = 2.3 Hz, 1H, 8-H), 7.27 (m, 1H, 3'-H), 7.37 (d, J₈₋₇ = 8.7 Hz, 1H, 7-H), 7.63 (m, 2H, 5'-H + 10-H), 7.93 (d, J₄₋₃ = 6.0 Hz, 1H, 4-H), 8.46 (m, 2H, 3H + 6'-H), 8.75 (s, 1H, 11-H).

5,6-Dimethyl-9-methoxy-1-(2-methylpyridin-4-yl)-6H-pyrido[4,3-b]carbazole (10)

A mixture of compound **9** (707 mg, 2 mmol), finely powdered dry potassium carbonate (500 mg), dimethyl carbonate (20 ml), dimethylformamide (2ml), and 18-crown-6-ether (2 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 from isopropyl acetate to give yellow crystals (588 g, 80%), mp 202-4°C. Anal. Calc'd for C₂₄H₂₁N₃O: C, 78.45; H, 5.76; N, 11.44. Found: C, 78.21; H, 5.63; N, 11.65. ¹H NMR (CDCl₃) δ: 2.72 (s, 3H, 2'-CH₃), 3.13 (s, 3H, 5-CH₃), 3.92 (s, 3H, 9-OCH₃), 4.16 (s, 3H, 6-CH₃), 7.11 (dd, J₈₋₇ = 8.7 Hz, J₈₋₁₀ = 2.2 Hz, 1H, 8-H), 7.29-7.34 (m, 2H, 7-H + 10-H), 7.50 (m, 1H, 3'-H), 7.67 (dd, J_{5'-6'} = 5.1 Hz, J_{5'-3'} = 1.8 Hz 1H, 5'-H), 8.02 (d, J₄₋₃ = 6.0 Hz, 1H, 4-H), 8.52 (d, J₄₋₃ = 6.1 Hz, 1H, 3-H), 8.74 (d, J_{5'-6'} = 5.0 Hz, 1H, 6'-H), 8.82 (s, 1H, 11-H).

5,6-Dimethyl-9-hydroxy-1-(2-methylpyridin-4-yl)-6H-pyrido[4,3-b]carbazole (5)

A mixture of compound **10** (367 mg, 1 mmol) and hydrobromic acid (48%) was heated at reflux under stirring for 3 h. After evaporation to dryness, the residue was taken up in water. The resulting mixture was basified with concentrated ammonia and extracted with methylene chloride, then dried over magnesium sulfate. After evaporation of the solvent, the solid was recrystallized from methylene chloride to give yellow crystals (157 mg, 44.5%), mp 279-280°C. Anal. Calc'd for C₂₃H₁₉N₃O: C, 78.16; H, 5.42; N, 11.89. Found: C, 77.97; H, 5.66; N, 11.73. ¹H NMR (DMSO-d₆) δ: 2.70 (s, 3H, 2'-CH₃), 3.11 (s, 3H, 5-CH₃), 4.13 (s, 3H, 6-CH₃), 7.11 (dd, J₈₋₇ = 8.7 Hz, J₈₋₁₀ = 2.2 Hz, 1H, 8-H), 7.25 (m, 1H, 3'-H), 7.46 (d, J₈₋₇ = 8.7 Hz, 1H, 7-H), 7.60 (m, 2H, 10-H + 5'-H), 7.85 (d, J₄₋₃ = 6.1 Hz, 1H, 4-H), 8.05 (d, J₄₋₃ = 6.0 Hz, 1H, 3-H), 8.50 (d, J_{5'-6'} = 5.0 Hz, 1H, 6'-H), 8.70 (s, 1H, 11-H).

Biological Studies

Bacterial strains: the study was carried out on *E. coli* O56 PCM 2372 (NCTC 9056) (PCM: Polish Collection of Microorganisms, Wrocław; NCTC: National Collection of Type Cultures, Central Public Health Laboratory, London). **Media:** YP broth (bactopectone Difco 1%, yeast extract Difco 1%, and NaCl 0.5%) was used as the liquid medium. Nutrient agar was purchased from the Factory of Sera and Vaccines in Warsaw. **Sera:** NBS (Normal Bovine Serum) was obtained from four healthy heifers untreated with antibiotics, allowed to clot, and separated in the cold. Sera were pooled and 1.0 ml aliquots were frozen at -70° C. The suitable volume of serum was thawed immediately before using. Each portion was used only once.

Preparation of bacterial culture

The *E. coli* O56 strains were growth in YP broth overnight, and then bacterial cells of the early exponential growth phase were transferred to fresh YP and incubated at 37° C for 1h. After incubation, the bacterial cells were centrifuged (4000 rpm for 20 min) and resuspended in 0.8% NaCl.

Effect of olivacine derivatives on growth of bacteria

The bacteria in 0.8% NaCl were mixed with the DMSO solution of olivacine derivatives and incubated at 37° C in a water bath After 0, 60, and 180 min. probes were collected, diluted, and plated on nutrient agar plates for 18 h at 37° C. The number of colony-forming units (c.f.u.) at time 0 was taken as 100%.

Bactericidal activity of normal bovine serum (NBS)

The bactericidal activity of NBS was determined according to the procedure of Doroszkiewicz et al. as described previously [9]. Briefly, the bacterial suspensions in saline were mixed with 12.5% NBS alone or with olivacine derivatives in a suitable concentration. Bacteria with serum were incubated in a water bath at 37° C and samples were collected at 0, 60 and 180 min., diluted, and cultured on nutrient agar plates for 18 h at 37° C. The number of colony-forming units (c.f.u.) at time 0 was taken as 100%.

References

- [1] Romaniewska A, Jasztold-Howorko R, Regiec A, Lis T, Kuduk-Jaworska J. Synthesis, Structure and Characterization of Olivacine Derivatives and their Platinum (II) Complexes. Eur. J. Inorg. Chem. 2003; 22: 4043-54.
- [2] Jasztold-Howorko R., Croisy A., Carrez D., Jaroszewicz I., Nasulewicz A., Pełczyńska M., Opolski A.. Synthesis, structure and cytostatic properties of new olivacine derivatives. Archive der Pharmazie, 2004, 337, 599-604.
- [3] Jasztold-Howorko R., Croisy A., Carrez D. New 1-substituted 9-hydroxy-5,6-dimethyl-6H-pyrido [4,3-b] carbazole derivatives and their cytotoxicity. Acta Poloniae Pharmaceutica in press.
- [4] Taylor P.W. Resistance of bacteria to complement. Virulence mechanism of bacterial pathogen. (J.A.Roth Ed.) Am. Soc. Microbiol. Washington. D. C. 1995
- [5] Taylor P.W. Complement-Mediated Killing of Susceptible Gram-Negative Bacteria: An Elusive Mechanism. Exp.Clin.Immunogenet. 1992; 9: 48-56.
- [6] Jasztold-Howorko R, Landras C, Pierré A, Atassi G, Guilbaud N, Kraus-Bertier L, Léonce S, Rolland Y, Prost J-F, Bisagni E. Synthesis and evaluation of 9-hydroxy-5-methyl-(and 5,6-dimethyl)-6H-pyrido[4,3-b]carbazole-1-N[(dialkylamino)alkyl]-carboxamides, a new promising series of antitumor olivacine derivatives. J. Med. Chem. 1994; 37: 2445-52.
- [7] Maftouh M, Besselivere R, Monsarrat B, Lesca P, Meunier B, Husson H.P, Paoletti C. Synthesis and cytotoxic activity of hydroxylated derivatives of olivacine in relation with biotransformation. J.Med.Chem. 1985; 28: 708-14.
- [8] Mielnik G, Gamian A, Doroszkiewicz W. Bactericidal activity of normal cord serum (NCS) against Gram-negative rods with sialic acid – containing lipopolysaccharides (LPS). FEMS Immun. Med. Microb. 2001; 31: 169-73.
- [9] Doroszkiewicz W. Mechanism of antigenic variation in *Shigella flexneri* bacilli. IV Role of lipopolisaccharides and their components in the sensitivity of *Shigella flexneri* 1b and its Lac⁺ recombinant to killing action of serum. Arch. Immunol. Ther. Exp. 1997; 45: 235-42.

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