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BIOLOGICAL ACTIVITY OF BACTERIAL LECTINS AND THEIR MOLECULAR COMPLEXES WITH HETEROCYCLIC BIS-ADDUCTS

A new convenient method for the preparation of heterocyclic bis-adducts: of imidazole, benzimidazole, uracile with 1,1,1-trifluoro-2-bromo-2-chloroethane is described. The reactions are catalysed by the 18-crown-6-complex.

The critical toxicity and antitumour activity of saprophytic strains Bacillus genus (B. subtilis 668 IMV and B. polymyxa 102 KSU) extracellular lectins were studies. It was discovered that these substances apply to a few toxic preparations and have a expression antitumour action on the tumours: Walker carcinosarcoma 256, Pliss' lymphosarcoma and Sarcoma 45.

The new molecular complexes were created with bacterial lectins and the same heterocyclic bis-adducts of unsubstituted benzimidazole and 6-methyluracile. A strongly antitumour effect of these complexes has been discovered: of growth relaxation of Pliss' lymphosarcoma tumour mass was 62.5 – 82.01 %.

K e y w o r d s: bacterial lectins, heterocyclic bis-adducts, biological activity.

Lectins are a group of proteins which interact with polysaccharides and glycoproteins by binding to specific carbohydrate residues; they are present in many plants, bacteria and inverterbrates [10]. In spite of the ubiquitous presence of lectins in nature (over 100 plants lectins have already been isolated and their carbohydrate binding properties at least partially characterized), relatively few have been characterized as specific for sialic acid [1, 8].

The ability for lectin secretion of saprophyte strains of sporeform bacteria of the *Bacillus* genus has been elegantly shown by us before [10]. These lectins have a specificity to sialic acid; they are inductors of γ-interferon and antitumour agents as well [6, 10]. It is known that the heterocyclic systems such as: uraciles, benzimidazole and imidazole are main components of antitumour drugs, anxiolytic agents or bactericides [3, 9, 11, 15].

The construction of new in principle medical preparations on the basis of the saprophytic strains of bacterial lectins and heterocyclic bis-adducts is rather promising.

In this paper we inform about the synthesis and characterization of heterocyclic bis-adducts, the toxicity and antitumour activity of bacterial lectins from *Bacillus* saprophytic strains (*B. subtilis* 668 IMV and *B. polymyxa* 102 KSU) and antitumour activity of new molecular complexes bacterial lectins with the same heterocyclic bis-adducts.

Materials and methods. The bis-adducts 3a – f of unsubstituted benzimidazole, imidazole and substituted uraciles with 1,1,1-trifluoro-2-bromo-2-chloroethane are obtained under phase-transfer conditions in alkaline medium [5]. The reactions are catalysed by the 18-crown-6-complex [16].

The method reported for the synthesis of bis-adducts 3a – f is based on the reactions which involve elimination of fluorine hydride, formation of the intermediate 1,1-difluoro-2-bromo-2-chloroethane which react with nucleophilic molecules [5].

The majority of the organic solvents (benzole, dimethylformamide, hexane, ethyl ether) employed in the present studies were distilled before use. Organic solvents were dried over anhydrous magnesium sulfate or metallic sodium.

IR spectra were recorded in a UR-20 spectrometer ("Charles Ceise Hena", Germany). The PMR (proton-magnitic resonance) spectra were recorded in DMCO-d6 (dimethylsulfoxide-deuterium 6) on a 200 MHz Bruker WP-200 ("Bruker", Switzerland) or Varian T-60 spectrometer ("Varian", USA).

The lectin preparations were obtained by treatment of culture liquid of two saprophytic strains *Bacillus* from Ukrainian Collection of Microorganisms of Institute of Microbiology and Virology (IMV): *B. subtilis* 668 IMV and *B. polymyxa* 102 KSU (Kiev State University); in future: lectin 668 and lectin 102 clarified by treatment with ammonium sulfate (70 % concentration of a saturated solution); the precipitate containing the lectin was dissolved in water, dialyzed against water and freeze dried as described elsewhere [6, 10].

The express-method of definition of LD_{50} by Prozorovsky et al. was used [12]. The investigation of the critical toxicity of bacterial lectins 668 and 102 was conducted on the white inbred mice with mass 20.0 ± 2.0 g and rats with mass 150.0 ± 20.0 g with standard food-ration. There were four ways of administration of physiological solutions of bacterial lectins: intramuscular, subcutaneous, intraperitoneal and intravenous.

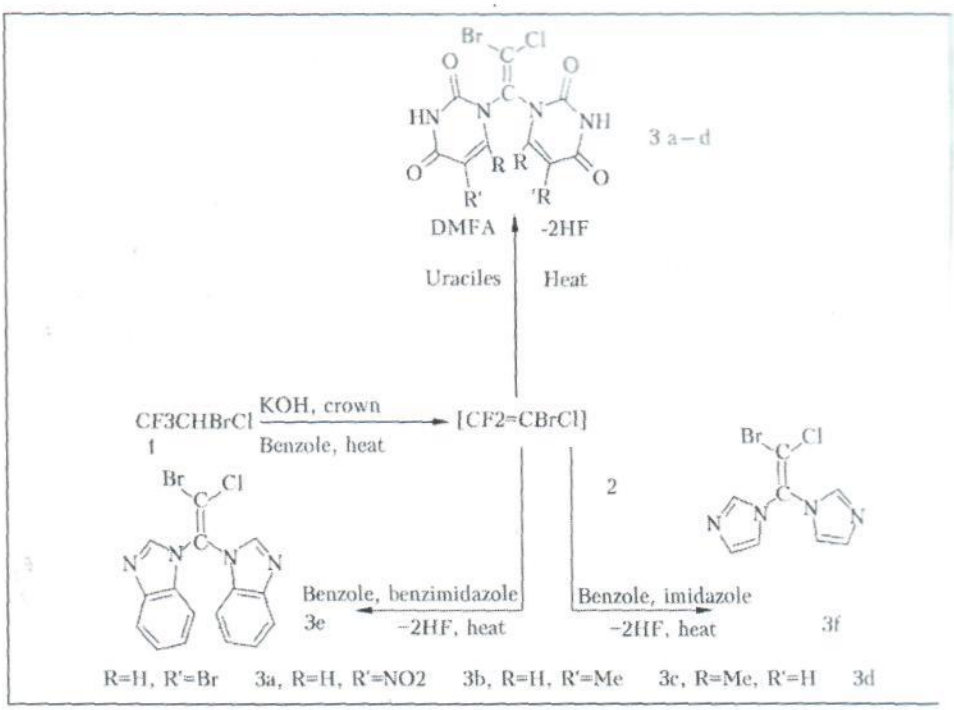
White inbred mice and rats (300 animals) and experimental models of tumour growth (Walker carcinosarcoma 256, Pliss' lymphosarcoma and Sarcoma 45) were used following published procedures [2, 4, 7]. The mixture of lectin 102 with heterocyclic bis-adducts 3c and 3e was introduced. The efficiency parameter: growth inhibition of the mass of these tumours was 50 % [4, 14].

There were 6 administrations of the physiological solutions of bacterial lectins and molecular complexes of heterocyclic bis-adducts with bacterial lectins (1 : 1) every other day. The dosage of the preparations corresponded to 1 / 4 – 1 / 6 of LD_{50}. There were six animals in group.

The results were assessed by standard methods of statistical analysis [13].

Results and their discussion. **1. C h e m i s t r y.** The general synthesis procedures used for the preparation are illustrated in Scheme. Analytical data for adducts 3a – f are shown in Table 1.

1.1. General procedure for the preparation of 1,1'-(2"-bromo-2"chloroethenyl) bis-(5-bromouracile) 3a.



The synthetic procedures used for bis-adducts preparation

A mixture of potassium hydroxide (3 g, 0,054 mol) and dibenzo-18-crow 6-ether (0,3 g, 0,054 mol) in 40 ml of dry benzole was heated under reflux 60 – 80 °C for 15 min.

The cooled solution was mixed with 1,1,1-trifluoro-2-bromo-2-chloroetha (4,68 g, 0,024 mol) in dry ethyl ether. A solution 1 of 1,1,1-trifluoro-2-brom 2-chloroethane and potassium complex of dibenzo-18-crown-6-ether was heat under reflux at 60 – 80 °C for 15 min. After that cooled solution 1 was mixed wi solution 2 (5-bromouracile (5.5 g, 0.0288 mol) in 40 ml of dry dimethylformamid and the heat under reflux at 60 – 80 °C for 12 h.

Tabl

Analytical data for adducts 3a—f

Adduct	Melting point, °C	Elemental analysis, %		
		C	H	N
		Found (calculated)		
3a	270 – 275	22.8 (23.13)	1.02 (0.77)	11.01 (10.78)
3b	290 – 295	27.0 (26.6)	1.03 (0.9)	17.98 (18.61)
3c	265	37.05 (36.9)	3.08 (2.58)	16.99 (16.55)
3d	280 – 285	37.0 (36.9)	3.05 (2.58)	15.97 (16.55)
3e	222 – 225	51.5 (51.43)	3.0 (2.7)	14.83 (14.9)
3f	107 – 110	34.89 (35.12)	2.50 (2.2)	20.39 (20.48)

The heated solution was filtered. The precipitate was washed with 30 ml of aqueous methyl alcohol, 10 ml of ethyl ether, and the solvent removed under reduced pressure. The adduct 3a is a cream-coloured solid (30 %); ν_{max}(KBr), cm^{-1}: 1750, 1710, 695, 550; δ H 7.66 ($2 \times C_{(6)}H$), 4.048 ($2 \times N_{(3)}H$ in H_2O).

1.2. 1,1'-(2"-bromo-2"-chloroethenyl)-bis-(5-nitrouracile) 3b. The adduct was prepared according to the general procedure. The adduct 3b is a yellow solid (56,5 %); ν_{max} (KBr), cm^{-1}: 1750, 1710, 1580, 690, 550; δH 10.32($2 \times N_{(3)}H$), 8.98 ($2 \times C_{(6)}H$).

1.3. 1,1'-(2"-bromo-2"-chloroethenyl)-bis-(5 (or 6)-methyluracile) 3c, 3d. The adducts were prepared according to the general procedure. There are white solids (26–30 %). The precipitates were washed with 25–30 ml of hexane, and the solvent removed under reduced pressure. For the adduct of 5-methyluracile, 3c: ν_{max} (KBr), cm^{-1}: 3000, 2800, 1750, 1710, 615, 515; δH 11.0 ($2 \times N_{(3)}H$), 7.25 ($2 \times C_{(6)}H$), 1.73 ($2 \times Me$). For the adduct of 6-methyluracile, 3d: ν_{max} (KBr), cm^{-1}: 3000, 2800, 1750, 1710, 615, 598, 515; δH 10.83 ($2 \times N_{(3)}H$), 5.31 ($2 \times C_{(5)}H$), 2.01 ($2 \times Me$).

1.4. 1-(2'-bromo-1', 1'-difluoro-2'-chloroethyl)-benzimidazole 3e and 1-(2'-bromo-1', 1'-difluoro-2'-chloroethyl)-imidazole 3f.

The adducts were prepared according to the general procedure. However, the solution 2 of heterocyclic compounds in dry benzole was prepared. The precipitate was washed with 30 ml of hot acetonitrile. The cooled solution was

Table 2

The median-lethal doses (LD_{50}) of bacterial lectins

Animals	The way of administration	LD_{50}, mkg/kg	
		Lectin 668	Lectin 102
Mice	Intramuscular	68 (46–101)	294 (210–318)
	Subcutaneous	71 (59–84)	248 (195–301)
	Intraperitoneal	89 (75–106)	200(154–246)
	Intravenous	37 (26–54)	Non determined
Rats	Intraperitoneal	71 (62–80)	60 (52–68)
	Intravenous	52 (45–59)	Non determined

Table 3

The antitumour activity of bacterial lectins and their molecular complexes with adducts 3e and 3c

Lectin	Dose, mkg/kg	Middle mass of tumour of control animals, g	Middle mass of tumour of experimental animals, g	% of growth inhibition of the tumour	Effective index	Spleen coefficient
Walker carcinosarcoma 256						
668	16	32.5 ± 0.21	15.5 ± 0.21	52.3	2.09	0.76
102	20	32.5 ± 0,21	16.2 ± 0.25	50.0	–	–
Pliss' lymphosarcoma						
668	16	42.0 ± 2.77	38.0 ± 3.22	9.0	1.1	1.1
102	20	42.0 ± 2.77	21.0 ± 0.80	50.0	1.1	–
102 + 3c	24	13.9 ± 1.93	2.5 ± 1.30	62.5	2.67	0.71
102 + 3e	35	13.9 ± 1.93	1.8 ± 0.09	82.01	5.56	0.71
Sarcoma 45						
668	17	16.14 ± 1.90	9.2 ± 0.17	42.9	1.75	1.04

removed. The precipitate was washed with 10 ml of a cold water, 10 ml of acetonitrile and the solvent removed under reduced pressure. The adducts 3e, 3f are yellow solids (48–53 %): ν_{max} (KBr), cm^{-1}: 3100, 3025, 1250, 1170 ; δH 8.91, 7.29 (2 × Ph) (for adduct 3e): ν_{max} (KBr), cm^{-1}: 3080, 3065, 690, 550; δH 8.914, 7.282 (2 × Ph), 7.27 (2 × H in (BrClCH-) (for adduct 3f).

2. Biology. The determination one of the main pharmacological indexes of medical preparations – the critical toxicity on two species of animals under different ways of administration was shown that the investigated lectins concern to low toxic substances (Table 2). We found that lectin 668 is more toxic (LD_{50} at different ways of administration was 37–89 mkg/kg) than lectin 102 (LD_{50} of this lectin was 200–294 mkg/kg).

The obtained parameters of the bacterial lectins toxicity show that LD_{50} under different ways of administration is similar. This evidences for the lectins' capability to pass quickly through the histo-haematic barriers.

A strong antitumour effect has been discovered for lectins 668 and 102 on Walker carcinosarcoma 256: growth inhibition of the tumour was 52.3 % and 50.0 %, accordingly (Table 3). Lectin 102 is more active as to Pliss' lymphosarcoma than lectin 668: growth inhibition of this tumour was as the efficiency parameter (50.0 %). The antitumour effect of lectin 668 on Sarcoma 45 was a bit lower (42.9 %). Percentage of primary recovery and destruction is "0".

The molecular complexes of lectin 102 with bis-adducts 3e and 3c were tested on Pliss' lymphosarcoma (Table 3). A strongly antitumour effect has been discovered for these complexes: growth inhibition of the tumour was 62.6 % and 82.01 % according. The complex of adduct 3e with lectin 102 is non-toxic and more activity as to this tumour.

Finally, we wish to stress that these primary investigations are rather promising. These results may be used for creation of new antitumour drugs.

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БІОЛОГІЧНА АКТИВНІСТЬ БАКТЕРІАЛЬНИХ ЛЕКТИНІВ ТА ЇХ МОЛЕКУЛЯРНИХ КОМПЛЕКСІВ З ГЕТЕРОЦИКЛІЧНИМИ БІС-АДУКТАМИ

Р е з ю м е

Описано новий препаративний метод одержання гетероциклічних біс-адуктів – імідазолу, бензимідазолу, урацилів та 1,1,1-трифтор-2-бром-2-хлоретану при міжфазовому каталізі 18-краун-6-комплексом.

Вивчено гостру токсичність та протипухлинну активність позаклітинних лектинів сапрофітних штамів бактерій роду *Bacillus* (*B. subtilis* 668 ІМВ та *B. polymyxa* 102 КДУ). Встановлено, що ці речовини відносяться до малотоксичних препаратів і мають виражену протипухлинну дію у відношенні карциносаркоми Уокера 256, лімфосаркоми Пліса та саркоми 45.

Створено нові молекулярні комплекси бактеріальних лектинів з деякими гетероциклічними біс-адуктами – похідними незаміщеного бензимідазолу та 6-метилурацилу. Виявлено високий протипухлинний ефект цих комплексів: гальмування росту лімфосаркоми Пліса становило 62,5–82,01 %.

К л ю ч о в і с л о в а: бактеріальні лектини, гетероциклічні біс-адукти, біологічна активність.

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БИОЛОГИЧЕСКАЯ АКТИВНОСТЬ БАКТЕРИАЛЬНЫХ ЛЕКТИНОВ И ИХ МОЛЕКУЛЯРНЫХ КОМПЛЕКСОВ С ГЕТЕРОЦИКЛИЧЕСКИМИ БИС-АДДУКТАМИ

Р е з ю м е

Описан новый препаративный метод получения гетероциклических бис-аддуктов – имидазола, бензимидазола, урацилов и 1,1,1-трифтор-2-бром-2-хлорэтана при межфазном катализе 18-краун-6-комплексом.

Изучена острая токсичность и противоопухолевая активность внеклеточных лектинов сапрофитных штаммов бактерий рода *Bacillus* (*B. subtilis* 668 ИМВ и *B. polymyxa* 102 КГУ). Установлено, что эти вещества относятся к малотоксичным препаратам и обладают выраженным противоопухолевым действием в отношении карциносаркомы Уокера 256, лимфосаркомы Плисса и саркомы 45.

Созданы новые молекулярные комплексы бактериальных лектинов с некоторыми гетероциклическими бис-аддуктами – производными незамещенного бензимидазола и 6-метилурацила. Выявлен сильный противоопухолевый эффект этих комплексов: торможение роста лимфосаркомы Плисса составляло 62,5–82,01 %.

К л ю ч е в ы е с л о в а: бактериальные лектины, гетероциклические бис-аддукты, биологическая активность.

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