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New molecular complexes of heterocyclic bis-adducts with bacterial lectins: synthesis and structure–activity relationship studies

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Abstract

A strongly antitumour effect has been discovered for lectins of Bacillus bacteria [*Bacillus subtilis* 668(1 + 2)IMV, *Bacillus polymyxa* 102(1 + 2) KGU] and for their molecular complexes with some heterocyclic bis-adducts of unsubstituted benzimidazole and 6-methyluracile for the first time. These were tested on the tumours: Lymphosarcoma Plissa, Sarcoma 45, Carcinosarcoma Yokera 256. A new convenient method for the preparation of the heterocyclic bisadducts of imidazole, benzimidazole, uraciles with 1,1,1-trifluoro-2-bromo-2-chloroethane is described. The reactions are catalysed by the 18-crown-6-complex.

Keywords: Lectins; Uracile derivatives; Antitumour activity

1. Introduction

Heterocyclic systems such as: uraciles; benzimidazole; imidazole; are main components of antitumour drugs, anxiolytic agents or bactericides [1–4]. The bis-adducts 3a–f of unsubstituted benzimidazole, imidazole and substituted uraciles with 1,1,1-trifluoro-2-bromo-2-chloroethane (1) are obtained under phase-transfer conditions in alkaline medium. The reactions are catalysed by the 18-crown-6-complex [5]. 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-2chloroethene (2) which reacts with nucleophilic molecules [6]. The general synthetic procedures used for their preparation are illustrated in Scheme 1. Analytical data for adducts 3a–f are shown in Table 1.

In this paper we report the synthesis, characterisation and antitumour activity of the molecular complexes of heterocyclic bis-adducts 3c, 3e with bacterial lectin [*Bacillus polymyxa* 102(1 + 2) KGU]. A strongly antitumour effect has been discovered for lectins of *Bacillus* bacteria [*Bacillus subtilis* 668(1 + 2)IMV, *Bacillus polymyxa* 102(1 + 2) KGU] and for their molecular complexes with heterocyclic bis-adducts 3c, 3e for the first time. These were tested on the tumours: Lymphosarcoma Plissa; Sarcoma 45; Carcinosarcoma Yokera 256.

Lectins are a group of proteins which interact with polysaccharides and glycoproteins by binding to specific carbohydrate residues, and which are present in many plants, bacteria and invertebrates [7]. In spite of the ubiquitous presence of lectins in nature (over 100 plants lectins have already been isolated and their carbohydrate binding specificity at least partially characterized), relatively few have been characterized as specific for sialic acid [8–10]. The ability for lectin secretion of saprophit stammes of sporeform bacteria of the genus *Bacillus* has been elegantly shown by Kovalenko et al. [11]. These lectins are non-toxic and specific for sialic acid. They are inductors of γ-interferones and antitumour agents [11,12].

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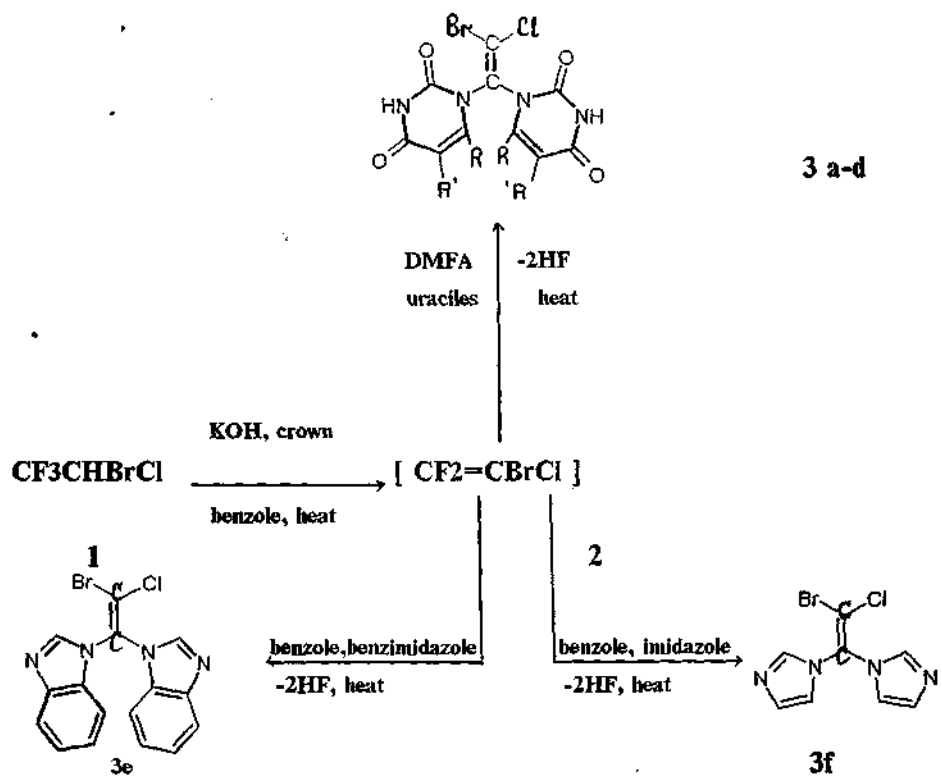

R=H, R′ =Br 3a, R=H, R′ = NO2 3b, R=H, R′ =Me 3c , R=Me, R′ =H 3d

Scheme 1.

2. Materials and methods

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 ^1H spectra were recorded in DMSO-d6 on a 200 MHz BrakerWP-200 ('Braker', Switzerland) or a Varian T-60 spectrometer ('Varian', USA).

The lectin preparations were obtained by treatment of *Bacillus* bacteria (*Bacillus subtilis*, *Bacillus polymyxa*) culture fluid clarified by treatment with ammonium sulphate (70% concentration of a saturated solution); the precipitate containing the lectin was dissolved in water, dialyzed against distilled water and freeze dried as described elsewhere [13].

The protein and carbohydrate composition has been determined by general methods. The amino acids composition has been determined using the aminoacid analyzer KLA ('Hitachi', Japan). The carbohydrate analysis has been determined (the polyol acetates) by vapor phase chromatography.

The white imbredical mice and rats (300 animals) and the experimental models of tumour growth [LS Plissa, Carcinosarcoma; Yokera 256 (W-256), Sarcoma 45] were used following published procedures [13–15]. The experimental tumours used for our investigation were obtained from the Bank of stammes of Oncological Centre of Academy of Medical Sciences of Russia. The experimental tumours were used for passage on experimental animals, program freezing and, after that, these

Table 1
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	285–287	38.00 (37.1)	3.08 (2.58)	16.99 (16.55)
3d	280–285	37.3 (37.1)	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 (2.21)	20.39 (20.48)

Table 2
Chemical composition of bacterial lectins 668 (1+2), 102 (1+2)

Lectin	% To weight composition	
	Albumen	Carbohydrate
668–1	76.0 ± 13.0	5.1 ± 0.1
668–2	53.7 ± 14.3	5.3 ± 0.2
102–1	75.5 ± 1.2	4.9 ± 0.3
102–2	56.5 ± 6.5	5.4 ± 0.5

Table 3
Amino acid content of bacterial lectins 668(1+2), 102(1+2) (mol%)

Amino acid	Lectin			
	668–1	668–2	102–1	102–2
Lysine	4.3	1.8	5.7	1.1
Histidine	4.8	7.9	1.9	9.2
Arginine	1.9	7.0	2.9	8.5
Asparagine	18.2	13.2	16.7	13.2
Glutamine	18.0	13.6	15.6	13.9
Threonine	5.3	11.4	4.4	13.4
Serine	6.1	11.5	7.0	10.5
Glycine	6.9	5.0	7.9	5.2
Alanine	4.5	4.2	8.0	4.8
Valine	12.8	11.5	11.9	10.4
Isoleucine	10.0	4.8	10.1	0.8
Leucine	1.9	3.8	2.0	2.0

Table 4
Monosaccharide content (% of common amount of saccharides)[a]

Monosaccharide	Lectin			
	668–1	668–2	102–1	102–2
Glucose	40.1	63.2	37.5	57.6
Galactose	54.0	34.0	55.2	40.1
Mannose	4.0	0	4.1	0
Arabinose	1.9	0	3.2	0
Rhamnose	Trace	1.7	Trace	1.5
Ribose	Trace	1.1	Trace	0.8

[a] A strong antitumour effect was obtained for the lectins 668 (1+2) and 102 (1+2) on Carcinosarcoma Yokera 256(W-256). Lectin 102(1+2) was found to be more active on Lymphosarcoma Plissa than lectin 668(1+2). The antitumour effect of Lectin 668(1+2) on Sarcoma 45 was not very high (42.9%). See Table 5.

were preserved in Bank of stammes of Institute of Pharmacology and Toxicology of Academy of Medical Sciences of Ukraine.

Lymphosarcoma Plissa was obtained on imbredical rat which received 3,3-dichlorobezidine [13]. The efficiency parameter [% of growth relaxation of Lymphosarcoma Plissa, (volume and mass)] is $\geqslant 50\%$. Carcinosarcoma Yokera 256 (W-256) was developed as spontaneous generation of adenocarcinoma of milk-gland of pregnant rat [16]. The efficiency parameter [% of growth relaxation of Carcinosarcoma Yokera 256(W-256), mass] is $\geqslant 50\%$. Sarcoma 45 was induced on imbredical rat during the hypodermic introduction of dimethylbenzoantracene [17].

The efficiency parameter (% of growth relaxation of Sarcoma 45, mass) is $\geqslant 50-70\%$.

The results were assessed by standard methods of statistical analysis [18].

3. Experimental

3.1. Chemistry

3.1.1. General procedure for the preparation of 1,1′(2″-bromo-2″-chloroethenyl)-bis-(5-bromouracile) 3a

A mixture of potassium hydroxide (3 g, 0.054 mol) and dibenzo-18-crown-6-ether (0.3 g, 0.054 mol) in 40 ml of dry benzole was heated under reflux at 60–80°C for 15 min. The cooled solution was mixed with 1,1,1-trifluoro-2-bromo-2-chloroethane (4.68 g, 0.024 mol) in dry ethyl ether. A solution I of 1,1,1-trifluoro-2-bromo-2-chloroethane and the potassium complex of dibenzo 18-crown-6-ether was heated under reflux at 60–80°C for 15 min. After that the cooled solution I was mixed with solution II [5-bromouracile (5.5 g, 0.0288 mol) in 40 ml of dry dimethylformamide] and then heated under reflux at 60–80°C for 12 h. The heated solution was filtered. The precipitate was washed width 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; δH 7.66 ($2 \times C_{(6)}H$), 4.048 ($2 \times N_{(3)}H$ in H_2O).

3.1.2. 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} (RBr) (cm^{-1}) 1750, 1710, 1580, 690, 550; δH 10.32 ($2 \times N_{(3)}H$), 8.98 ($2 \times C_{(6)}H$).

3.1.3. 1,1′-(2″-Bromo-2″-chloroethenyl)-bis-(5 (or 6)-methyluracile) 3c, 3d

The adducts were prepared according to the general procedure. They 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 ($2 \times C_{(6)}H$), 2.01 ($2 \times$ Me).

Table 5
The antitumour activity of lectins 668(1+2), 102(1+2) and their molecular complexes with bis-adducts 3e, 3c[a]

Lectin	Dose (mg kg^{-1})	Middle mass of the tumour of the control animals (g)	Middle mass of the tumour of the experimental animals (g)	Tumours	% Of growth relaxation of the tumour	Index of effectivity	Spleen coefficient *t*
CY 256[b]							
668	16	32.5 ± 0.21	15.5 ± 0.21		52.3	2.09	0.76
102	500	32.5 ± 0.21	15.5 ± 0.21		50.0	–	–
LP[c]							
668	16	42.0 ± 2.74	38.0 ± 3.22		9.0	1.1	1.1
102	400	42.0 ± 2.74	38.0 ± 3.22		50.0	–	–
102+3c	24	13.9 ± 1.93	1.8 ± 0.09		62.5	2.67	0.71
102+3e	35	13.9 ± 1.93	2.5 ± 1.3		82.01	5.56	0.71
S 45[d]							
668	17	16.14 ± 1.9	9.2 ± 0.17		42.9	1.75	1.04

[a] The molecular complexes of bis-adducts 3e, 3c with lectin 102(1+2), 1:1, were studied on Lymphosarcoma Plissa. A strong antitumour effect was observed for complexes for the first time. The complex of adduct 3e with Lectin 102 (1+2) is less toxic (MPD 35 mg kg^{-1}) and more active on the tumour. Finally, it showy stressed that these investigations are very preliminary. These results may provide a basis for creation of new antitumour drugs.

[b] Carcinosarcoma Yokera 256.

[c] Lymphosarcoma Plissa.

[d] Sarcoma 45.

3.1.4. 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 II of heterocyclic compounds in dry benzole was prepared. The precipitate was washed with 30 ml of hot acetonitrile. The cooled solution was filtered. The precipitate was washed with 10 ml of 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 8.963 (2 × CH), 7.766, 7.301 (2 × Ph) (for adduct 3e); ν_{max} (KBr) (cm^{-1}) 3080, 3065, 690, 550; δH 8.85(2 × CH), 6.42, 6.414 (4 × CH) (for adduct 3f).

3.2. Biology

Bacterial lectins of two cultures [*Bacillus subtilis* 668 (1 + 2)IMV, *Bacillus polymyxa* 102 (1 + 2) KGU] were obtained from culture liquid of saprophit (harmless for man and animals) culture of *Bacillus* bacteria. One percent solutions of each lectin in physiological pure medium have been used for the determination of composition (presence and quantity of proteins, carbohydrates) of these lectins.

All isolated males of imbredical mice and rats were provided with standard food ration in all groups with the same control. The quantity of animals in each group was six. Minimum mass of mice body was 20 ± 3–4 g, and of rats was 90–120 g. The age of the mice was 2–3 months, the age of the rats was 1.5–2 months. Percentage primary recovery and destruction is '0'. Method of killing—decapitation, redosage of ethyl ether. The method of removal of the experimental tumours is surgical. The efficiency parameter [% of growth relaxation of the tumour (volume and mass)] was counted by the formula [17]:

$$\frac{\text{(middle data of tumour growth in control)} - \text{(middle data of tumour growth in experimental group)}}{\text{(middle data of tumour growth in control)}} \times 100\%$$

There were six introductions of the physiological solutions of bacterial lectins and molecular complexes of heterocyclic bis-adducts with bacterial lectins every other day. The dosage of the preparations corresponded to 1/4–1/6 of the LD_{50}. The express-method of definition of LD_{50} by V.B. Prozorovsky was used [19].

The main control data are: middle mass of the tumour of the control animals (g); middle mass of the tumour of the experimental animals (g); % of growth relaxation of the tumour; index of effectivity; spleen coefficient.

4. Results and discussion

The chemical composition of extracellular sialospecific lectins of Bacillus species [*Bacillus subtilis* 668(1 + 2)IM4V, *Bacillus polymyxa* 102 (1 + 2)KGU] have been studied. It has been shown that these biopolymers are glycoproteins distinguished by their quantitative and qualitative proteins and carbohydrate composition. It has been established that certain bacteria can synthesise two extracellular lectins; one of which is an N-asparagine-bound glycoprotein, whilst the other is an O-serine/threonine-bound glycoproteins.

Chemical compositional data for the bacterial lectins [*Bacillus subtilis* 668(1 + 2)KGU, *Bacillus polymyxa* 102 (1 + 2) KGU] are shown below in Tables 2–5.

5. Conclusions

New molecular complexes (heterocyclic bis-adducts 3c, 3e with Bacterial lectin [*Bacillus polymyxa* 102 (1 + 2) KGU) and Bacterial lectins (*Bacillus subtilis* 668 (1 + 2), *Bacillus polymyxa* 102 (1 + 2)] were prepared and tested for their antitumour activity on certain tumours (Lymphosarcoma Plissa, Sarcoma 45, Carcinosarcoma Yokera 256). A new convenient method for the preparation of heterocyclic bis-adducts 3a–f of imidazole, benzimidazole, uraciles with 1,1,1-trifluoro-2-bromo-2-chloro-ethane is described. The reactions are catalysed by the 18-crown-6-complex. The lectin preparations were obtained by treatment of *Bacillus bacteria* species series [*Bacillus subtilis* 668(1 + 2) IMV, *Bacillus polymyxa* 102 (1 + 2) KGU] culture fluid clarified with ammonium sulphate (70% concentration of a saturated solution).

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