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SEARCH OF ANTITUMOUR MEDICAL DRUGS BY WAY OF CREATION OF NEW ANTIMETABOLITES OF PYRIMIDINES CHANGE – BIS-DERIVATIVES OF 5(6)-SUBSTITUTED URACILES AND THEIR ADDUCTS WITH BACTERIAL LECTINS

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Abstract

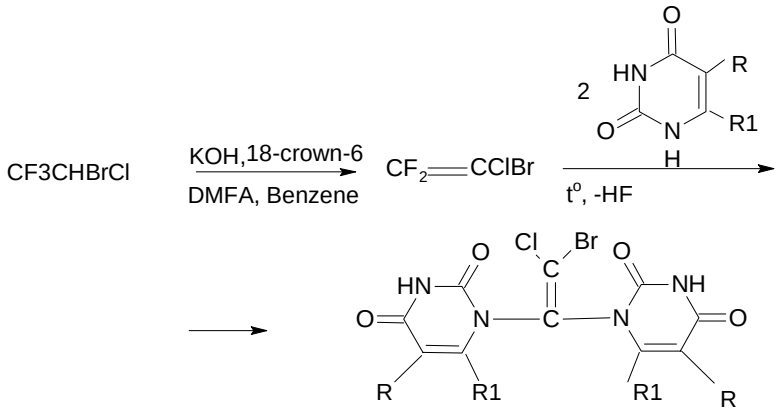
A new convenient method for the preparation of heterocyclic bis-adducts of 5(6)-substituted uraciles with ftorotan (1,1,1-threefluoro-2-bromo-2-chloroethane) is described. The reactions are catalyzed by the 18-crown-6-complex. The structure of synthesized compounds has been confirmed by data of elemental analysis, IR- and IH-spectra. The new molecular complexes were created on the base of bacterial lectins and some of heterocyclic bis-adducts of 5(6)-substituted uraciles, which synthesized. The critical toxicity and antitumour activity of new bis-adducts, saprophytic stammes Bacillus genus (B. subtilis 668 IMV and B. polymyxa 102 KGU) extracellular lectins and their molecular complexes were studies. It was discovered that these substances apply to little and moderate toxic preparations and has an expression antitumour action on the tumours: Lymphosarcoma Plissa and Sarcoma 45. A strongly antitumour effect of bis-adduct of 5-fluorouracile, bacterial lectins and their molecular complexes (bis-adduct of 5-fluorouracile + B. subtilis 668 IMV or B. polymyxa 102 KGU) has been discovered: growth relaxation of the Lymphosarcoma Plissa tumour mass was 50,0 -75,3%, of the Sarcoma 45: 16,9-81,1%.

Key words: *5-fluorouracile, bacterial lectins, heterocyclic bis-adducts, antitumour effect.*

One of the perspective ways of the search of new antitumour medical drugs are the creation of new antimetabolites of pyrimidines and purines change which will influence on structure and functions of nucleonic acids. The current of these investigations confirmed by a lot of scientific works (Preobrazenskaya M.N. 1973; Noordhuis P., Holwerda U. 2004; Adjei A. 1999; Larionoff L.F. 1962). Heterocyclic systems such as unsubstituted and 5(6)-substituted uraciles are main components of antitumour drugs (Sanyal U., Mitra S., Pal P., Chakraborty S.K. 1986; Bloknin N.N., Perevodchikova N.I. 1984). It is known tumors are using molecules of uraciles more active than normal cells. Therefore 5-fluorouracile (5-FU) and its derivatives will substrates and/or inhibitors of ferments and will swallow up by tumors cells. From other side molecules of 5(6)-fluoro(halogen)substituted uraciles can to carry out the role of fluoro- or halogen containing sintones at the organic synthesis therefore these compounds are very actually for creation of new original biological active molecules. The bis-adducts I-V of 5(6)-substituted uraciles with ftorotan (1,1,1-threefluoro-2-bromo-2-chloroethane) are obtained under phase-transfer conditions in alkaline medium (Sanyal U. et al. 1986). The reactions are catalyzed by the 18-crown-6-complex. The method reported for the synthesis of bis-adducts I-V is based on the reactions, which involve elimination of fluorine hydride, formation of the intermediate 1,1-difluoro-2-bromo-2-chloroethene, which reacts with nucleophilic molecules (Gerus I. et al. 1989; Welchinskaya E., Kuzmenko I., Ilchenko A. 1997). This reaction helps to find a new strategy for synthesis of selective polyfunctional molecules with chemical structure which permissible for

introduction of new pharmacophores. The general synthetic procedures used for their preparation are illustrated in Scheme 1.

Scheme1. The general synthetic procedures used for preparation of compounds I–V.



I - V

R = H, R1 = CH3 (I); R = CH3, R1= H (II); R= F, R1 = H (III); R= Br, R1 = H (IV); R= NO2, R1 = H (V)

Analytical and spectral data for bis-adducts I-V are shown in Table 1.

Table1. Analytical and spectral data for bis-adducts I-V.

Bis-ad-duct	Melting point (o C)	Elemental analysis (%)			IH spectra
		C	H	N	(DMCO-d6), δ, TMS,
		Found (calculated)			m.d. (J, Hz)
I	286-287	38,08 (37,1)	3,2 (2,58)	14,8 (14,38)	2,004 (6H, s., 2CH3); 5,313 (2H, s., 2C(5)H); 10,832 (2H, d., 2N(3)H, JH,H4 9,6 Hz).
II	265-268	37,60 (37,1)	3,08 (2,58)	14,53 (14,38)	1,712 (6H, d., J2H,H 5 Hz, 2CH3); 7,229 (2H, d., J2H,H 5 Hz, 2C(6)H); 10,7 (2H, s., 2N(3)H).
III	238-240	30,08 (30,21)	1,15 (1,13)	13,78 (14,09)	4,532 (2H, s., 2N(3)H); 7,447 (2H, d., 2C(6)H).
IV	270-275	22,8 (23,13)	1,02 (0,77)	11,01 (10,78)	4,048 (2H, s., 2N(3)H in H2O); 7,66 (2H, s., 2C(6)H).
V	290-295	26,67 (26,59)	1,02 (0,89)	17,79 (18,60)	*8,861 (2H, s., 2C(6)H); 10,226 (2H, s., 2N(3)H).*

In this paper we report the synthesis, characterization, toxicity and antitumour activity of the heterocyclic bis-adducts I-V, bacterial lectins (*Bacillus subtilis 668 IMV and Bacillus polymyxa 102 KGU)* and their molecular complexes with bacterial lectins (*Bacillus subtilis 668 IMV and Bacillus polymyxa 102 KGU)*. A strongly antitumour effect has been discovered for heterocyclic bis-adduct III, for lectins (*Bacillus subtilis 668 IMV and Bacillus polymyxa 102 KGU)* and for their molecular complexes with bis-adduct III for the first time. These were tested on the tumours: Lymphosarcoma Plissa (LS Plissa); Sarcoma 45. Lectins are a group of proteins interacts with polysaccharides and glycoproteines by binding to specific carbohydrate residues (Ahmed H. et al. 1986). The ability for lectins secretion of saprophit stammes of sporeform bacteria of the genus *Bacillus* has been elegantly shown by (Podgorsky V.S., Kovalenko E.A., Simonenko L.A. 1992). These lectins are non-toxic and specific for sialic acid. They are inductors of γ-interferones and antitumour agents (Podgorsky V.S., Kovalenko E.A., Simonenko L.A. 1992; Kovalenko E.A., Koltakova N.V., Getman E.I. 1991).

MATERIALS AND METHODS.

Objects of investigations: new heterocyclic bis-adducts of 5(6)-substituted uraciles with ftorotan (1,1,1-threefluoro-2-bromo-2-chloroethane), bacterial lectins (*Bacillus subtilis 668 IMV and Bacillus polymyxa 102 KGU)*, molecular complexes of bis-adducts which synthesized with bacterial lectins. The majority of the organic solvents (benzene, dimethylformamide (DMFA), hexane, ethyl ether, acetonitrile) employed in the present studies were distilled before use. Acetonitrile was dried during the distillation over P2O5. Organic solvents (benzene, DMFA, hexane, ethyl ether) were dried over anhydrous magnesium sulfate or metallic sodium. The structure of synthesized compounds has been confirmed by data of elemental analysis. The purity has been tested by method of thin-layer and gas-liquid chromatography. IR spectra were recorded in a UR-20 spectrometer ("Charles Ceise Hena", Germany). The IH spectra were recorded in DMCO-d6 with TMS as internal standard on a 200-132 MHz Bruker WP-200 ("Bruker", 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 (Podgorsky V.S., Kovalenko E.A., Simonenko L.A. 1992). The white imbredical mice (300 animals) and the experimental models of tumour growth (LS Plissa, Sarcoma 45) were used following published procedures (Larionoff L.F. 1962; Bloknin N.N., Perevodchikova N.I. 1984; Pliss G.V. 1961). The experimental tumours used for our investigations were obtained from the Banc of stammes of Oncological Center of Academy of Medical Sciences of Russian Federation. The experimental tumours were used for passage on experimental animals, program freezing and, after that, these 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-dichlorobenzidine (Bloknin N.N., Perevodchikova N.I. 1984). The efficiency parameter [% of growth relaxation of LS Plissa, (volume and mass)] is $\geq$50%. Sarcoma 45 was induced on imbredical rat during the hypodermic introduction of dimethylbenzoantracene (Sophiyena Z.P. et al. 1980). The efficiency parameter [% of growth relaxation of Sarcoma 45, (mass)] is $\geq$ 50-70%. The results were assessed by standard methods of statistical analysis (Senetlyaev D. 1971).

Experimental part.

1. Chemistry

General procedure for the preparation of N(1),N(1')-(2''-bromo-2''-chloroethenyl)-bis-(6-methyluracile) (I). Preparing of Solution ***1***. A mixture of potassium hydroxide (0.25 g, 0.0044 mol) and dibenzo-18-crown-6-ether (0.025 g, 0.0044 mol) in 20 ml of dry benzene was heated under reflux at 60-80 0C for 15 minutes. The cooled solution was mixed with ftorotan (0.87 g, 0.0044 mol) in 20 ml of dry ethyl ether. *Preparing of Solution* ***2***. A mixture of 6-methyluracile (1.11 g, 0.0088 mol) in 40 ml of dry DMFA was heated at 60 0C for 15 minutes. After that cooled Solution ***1*** was mixed with hot Solution ***2*** (6-methyluracile in dry DMFA) and then heated under reflux at 60-80 0C for 1 h, filtered before cooling, cooled and the solvents removed under reduced pressure. The precipitate was washed with 30 ml of mixture ethyl ether – hexane (1:1), dried under pressure. Adduct I is a crystalloid yellow-colored solid. Yield 1,85 g (43%). C12H10BrClN4O4. νmax (KBr), cm-1: 515, 550, 690, 850 (C-Hal); 960-970 (trans-C=C-); 1710, 1750 (C=O); 2800-3000 (CH3). *N(1),N(1')-(2''-bromo-2''-chloroethenyl)-bis-(5-fluorouracile) (III).* Adduct III was prepared according to the general procedure on the base of ftorotan (0.87 g, 0.0044 mol) and 5-fluorouracile (1.15 g, 0.0088 mol). Adduct III is a crystalloid cream-colored solid. Yield 1,75 g (50%). C10H4BrF2ClN4O4. νmax (KBr), cm-1: 510, 550, 690 (C-Hal); 1150, 1210 (C-F); 1735, 1750 (C=O). *N(1),N(1')-(2''-bromo-2''-chloroethenyl)-bis-(5-bromouracile) (IV).* Adduct IV was prepared according to the general procedure on the base of ftorotan (0.87 g, 0.0044 mol) and 5-bromouracile (1.67 g, 0.0088 mol). Adduct IV is a crystalloid cream-colored solid. Yield 1,36 g (30%). C10H4Br3ClN4O4. νmax (KBr), cm-1: 550-695 (C-Hal), 1710, 1750 (C=O). *N(1),N(1')-(2''-bromo-2''-chloroethenyl)-bis-(5-nitrouracile) (V).* Adduct V was prepared according to the general procedure on the base of ftorotan (0.87 g, 0.0044 mol) and 5-nitrouracile (1.38 g, 0.0088 mol). Adduct V is a crystalloid cream-colored solid. Yield 2,24 g (56,5%). C10H4BrClN6O8. νmax (KBr), cm-1: 550-690 (C-Hal), 1710, 1750 (C=O).

2. Biology

More active producents of bacterial lectins (*Bacillus subtilis 668 IMV and Bacillus polymyxa 102 KGU)* from Ukrainian Collection of Microorganisms of Institute of Microbiology and Virology (IMV) of NAS of Ukraine were used for preparation of molecular complexes on the base of new bis-adducts and bacterial lectins. Bacterial lectins of two cultures (*Bacillus subtilis 668 IMV and Bacillus polymyxa 102 KGU)* were obtained from culture liquid of saprophit (harmless for man and animals) culture of *Bacillus* bacteria (Podgorsky V.S., Kovalenko E.A., Simonenko L.A. 1992). Molecular complexes of bis-adducts and bacterial lectins were obtained by mixing of two components at physiological solution, 1:1. All isolated males of imbredical mice were provided with standard food ration in all groups with the same control. The quantity of animals in each group was ten. Minimum mass of mice body was 20±3-4 g. The age of the mice was 2-3 months. Percentage primary recovery and destruction was "0". Method of killing was decapitation, redosage of ethyl ether. The method of removal of the experimental tumours was sufginal. The efficiency parameter [% of growth relaxation of the tumour (volume and mass)] was counted by the formula (Sophiyena Z.P. et al. 1980):

(middle data of tumour growth in control) - (middle data of tumour growth in experimental group) /(middle data of tumour growth in control) x 100%.

There were six introductions of the physiological solutions of bis-adducts, bacterial lectins and their molecular complexes every day. The way of introduction was hypodermic. Preparation –standard was 5-fluorouracile. The dosage of the preparations corresponded to 1/4-1/6 of the LD50. The express-method of definition of LD50 for bis-adducts, bacterial lectins and their molecular complexes by Prozorovskiy V.B. et al. was used (Prozorovskiy V.B., Prozorovskiy V.P., Demchenko V.M. 1978).

The main control data were: LD50; 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.

RESULTS AND DISCUSSION.

Bis-adducts, which synthesized on the base of 5-methyluracile (II), 5-fluorouracile (III), 5-bromouracile (IV) and ftorotan as more relatives to 5-fluorouracile were choice for biological investigations. Data of investigations of toxicity of bis-adducts II, III, IV confirmed their little toxicity: LD50 of bis-adducts II and IV are 515 mg/kg and 415 mg/kg, corresponding. Bis-adduct III has little toxicity too: LD50 = 125 mg/kg; it is 4,12 and 3,32 times as much than toxicity of bis-adducts II and IV. LD50 of preparation-standard – 5-fluorouracile is 375 mg/kg. Bacterial lectins are more toxically than bis-adducts II, IV: *Bacillus subtilis 668 IMV* has moderate toxicity (LD50 = 89 mg/kg), *Bacillus polymyxa 102 KGU* has little toxicity (LD50 = 248 mg/kg). Molecular adducts of bis-adducts II, III, IV with *Bacillus subtilis 668 IMV* or *Bacillus polymyxa 102 KGU* has little toxicity too: LD50 from 635 to 137 mg/kg (Table 2).

Table2. Data of toxicity for bis-adducts II, III, IV; bacterial lectins and their molecular complexes.

№	Bis-adduct	LD50, mg/kg	Bacterial lectin	LD50, mg/kg	Molecular complex	LD50, mg/kg
1.	II	515	Lectin 102	248	II + Lectin 102	335
2.	III	125	Lectin 668	89	III + Lectin 668	137
3.	IV	415	Lectin 102	248	IV + Lectin 102	635
4.	5-FU, control	375				

Bis-adduct III – derivative of 5-fluorouracile and ftorotan as structural analogous of antitumour drug 5-fluorouracile, bacterial lectins (*Bacillus subtilis 668 IMV, Bacillus polymyxa 102 KGU*) and their molecular complexes were choice for investigations of antitumour activity on tumour growth (LS Plissa, Sarcoma 45). A strongly antitumour effect of bis-adduct III was registered on LS Plissa tumour: % of growth relaxation of the tumour was 75,3. A strongly antitumour effects of bacterial lectins were registered too: for *Bacillus polymyxa 102 KGU* on LS Plissa tumour: % of growth relaxation of the tumour was 50,0%; for *Bacillus subtilis 668 IM* on Sarcoma 45: 52,5%. Stability in antitumour activity, high % of primary recovery of animals were registered for both molecular complexes (Bis-adduct III + *Bacillus subtilis 668 IMV*; Bis-adduct III + *Bacillus polymyxa 102 KGU*) on LS Plissa and Sarcoma 45: % of growth relaxation of the tumour was from 62,8% to 81,1%. It is 1,14 times for LS Plissa and 4,46 times for Sarcoma 45 as much than antitumour activity of 5-fluorouracile (Table 3).

Table 3.Data of antitumour activity for bis-adduct III, bacterial lectins and their adducts.

Preparation	Dose, mg/kg	Middle mass of the tumour,g		Growth relaxation of the tumour, %
		control	experiment	
Lymphosarcoma Plissa				
Bis-adduct III	50,0	27,66	6,83	75,3*
Lectin 668	16,0	42,0±2,77	38,0±3,22	9,0
Lectin 102	20,0	42,0±2,77	21,0±0,18	50,0
Bis-adduct III + Lectin 668	24,0	13,9±1,93	1,8±0,09	62,8
5-FU, control				55,0 *
Sarcoma 45				
Bis-adduct III	30,0	62,7	52,12	16,9
Lectin 668	20,0	2,5±0,17	1,12±0,24	52,5
Bis-adduct III + Lectin 102	30,0	13,9±1,93	2,5±1,30	81,1
5-FU, control				18,4**

Note: *– death of one animal at experimantal group;

**– (Prozorovskiy V.B., Prozorovskiy V.P., Demchenko V.M. 1978)

CONCLUSIONS

A new convenient method with catalyzing by the 18-crown-6-complex for the preparation of heterocyclic bis-adducts of 5(6)-substituted uraciles with ftorotan (1,1,1-threefluoro-2-bromo-2-chloroethane) is described. The critical toxicity and antitumour activity of new bis-derivatives of uraciles, bacterial lectins (*Bacillus subtilis 668 IMV*, *Bacillus polymyxa 102 KGU*) and their adducts were studies. It was discovered that these substances apply to little and moderate toxic preparations and have an expression antitumour action on the tumours: Lymphosarcoma Plissa and Sarcoma 45. The new adducts of compounds and lectines were created on the base of bacterial lectins and derivative of 5-FU. A strongly antitumour effect of new adducts (derivative of 5-FU + *B. subtilis 668 IMV* or *B. polymyxa 102 KGU*) has been discovered: growth relaxation of the Lymphosarcoma Plissa tumour mass was 62,8%, of the Sarcoma 45: 81,1%. Derivative of 5-FU and its molecular adducts with bacterial lectins (*B. subtilis 668 IMV, B. polymyxa 102 KGU*) are perspective substances for further investigations as potential medical drugs with antitumour activity.

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