GLYCOCHEMISTRY AND ANTITUMOUR ACTIVITY OF ADDUCT OF BACTERIAL LECTINE AND 5-NITROURACILE

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Abstract

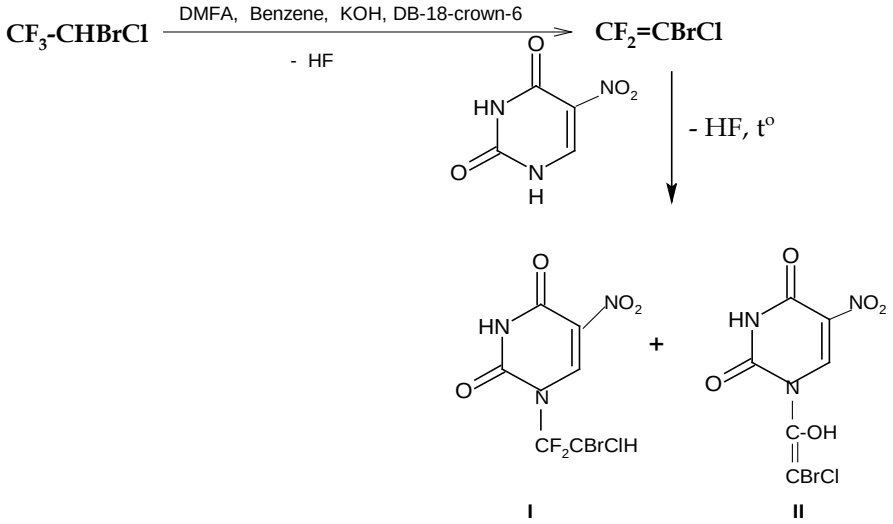
Problem of the treatment of man's cancer and search of the effective, with a little toxicity antitumour medical products, is one from the important task at the contemporaneous medicine and pharmaceutical chemistry. Chemical modification of molecular of 5-nitrouracile with next investigation of toxicity and antitumour activity of its new derivatives which synthesized is described. The structure of synthesized compounds has been confirmed by data of elemental analysis, IR- and ^{1}H-spectra. Physical-chemical, statistical pharmacological, toxicological methods were used. A strongly antitumour effect has been discovered for bis-derivative of 5-nitrouracile and for its adduct with Bacterial lectine 102 for the first time. A new convenient methods for the preparation of new mono- and bis-derivatives of 5-nitrouracile with 1,1,1-trifluoro-2-bromo-2-chloroethane (halothane) and l,l-diethylcarboxy-2-chloro-2-trifluoromethylethylene is described. The reactions are catalyzed by the DB-18-crown-6-complex. Bis-derivative of 5-nitrouracile and it's adduct with Bacterial lectine 102 were tested on the heterotransplantates of man's glioma cancer of brain (by Bogden's under capsule-method) and Carcinosarcoma Wokera 256. It is permits to consider the new bis-derivative of 5-nitrouracile and it's adduct with Bacterial lectine 102 as physiological active with a perspective investigation as potential antitumour drugs for treatment of man in future.

Keywords: *5-nitrouracile, 5-fluorouracile, bacterial lectins, antitumour effect, halothane*

Problem of the treatment of man's cancer and search of the effective, non-toxically antitumour medical products is one of important task at the modern medicine. Knowledge of cancer's cell's specifics and metabolism permits to plan the main direction of the chemical and biological investigations, to carry out purposeful synthesis of the potential drugs, to mark the possibility of its using at the practice of oncology as antitumour medical products. Medical drugs - heterocyclic derivatives (treatment of cancer of alimentary canal and other) at the arsenal of antitumour drugs took the important place as Abou - Gharbia et al. (1988), Alonso et al. (1984) and Perevodchikova et al. (2005) write. 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. Heterocyclic systems such as: 5(6)-substituted uraciles, pyrimidines; are main components of antitumour drugs, anxiolytic agents or bactericides as Adjei (1999), Barlow (1959), Anderson et al. (1992), Anttila et al. (1983), Benz et al. (1982), Longley et al. (2004) and Noordhuis et al (2004) write. Molecules of 5(6)-fluoric-(halogen)-substituted uraciles and its derivatives can to play a role of halogen containing syntones at organic synthesis, therefore these compounds are actively using for the building of original biological activity molecules. Besides that, halogen substituted groups and fragments to increase of it are soluble at the lipids. It is help to prepare medical drugs, which more effective (easiness in transportation inside the organism) as Au et al. (1979), Baba et al. (2000) and Yagupolskiy (1988) write. In this paper we report the synthesis, characterization, toxicity and antitumour activity of new mono- and bis-adducts of 5-nitrouracile and halothane or fluoric containing ethylene. It is necessary to accented that compounds of our investigation has heterocyclic fragment which are connected with remainder of molecule halothane - widely using at the surgical oncological practice (Brody, 1963; Brown, 1977). The derivatives of 5-nitrouracile and halothane I-III are obtained under phase-transfer conditions in alkaline medium. The reactions are catalyzed by DB-18-crown-6-complex. The method reported for the synthesis of adducts I-III is based on the reactions which involve elimination of fluorine hydride, formation of the intermediate l,l-difluoro-2-bromo-2-chloroethene, which reacts with nucleophilic molecules as (Gerus I. et al. 1989; Welchinskaya E.,

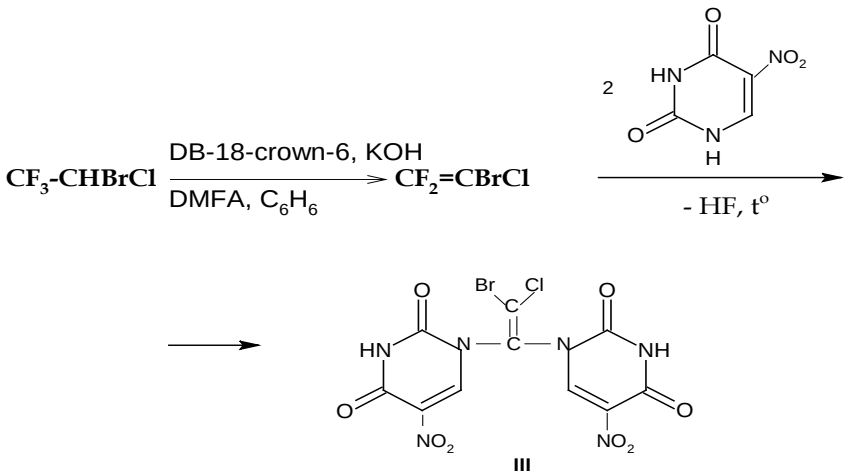
Kuzmenko I., Ilchenko A. 1997). Yagupolskiy (1988) writes. This reaction helps to find a new strategy for synthesis of selective polyfunctional molecules with chemical structure which permissible for introduction of new pharmacophores. New polyfunctional compound IV for using at the reactions with nucleophilic uracile was obtained. Original substituted 5-nitrouracile with fluoric containing ethylene group V is obtained at system of dry solvents DMFA-ethyl ester in presence of triethylamine anhydrous for the first time. The general synthetic procedures used for their preparation are illustrated in Schemes I-III.

Scheme I: Synthesis of new compounds with groups $-CF_2$-CHBrCl, -(HO)C=CBrCl (I, II)



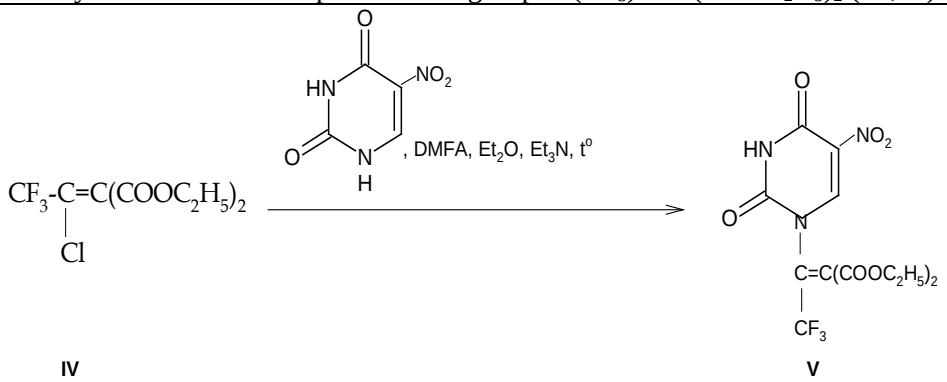
Source: Authors

Scheme II: Synthesis of new compound with group =C=CBrCl (III)



Source: Authors

Scheme III: Synthesis of new compounds with groups -$(CF_3)C=C(COOC_2H_5)_2$ (IV, V)



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

Table 1. Analytical and spectral data for compounds I-V.

Com-pound	Melting/ boiling point (°C)	Elemental analysis (%)	^{1}H spectra
		N	(DMCO-d_6), δ, TMS,
		Found (calculated)	m.d. (J, Hz)
I	269-271	12.54 (12.55)	5.65-5.68 (1H, $J^3_{H,F}$5.4 Hz, $J^2_{H,Cl(Br)}$0.8 Hz, $CF_2CHBrCl$), 7.24 (1H, $C_{(6)}H$), 10.57 (1H, $N_{(3)}H$)
II	264-267	13.43 (13.46)	7.24 (1H, $C_{(6)}H$), 10.57 (1H, $N_{(3)}H$), 10.98 (1H, OH)
III	290-295	18.59 (18.61)	8.86 (2H, $2C_{(6)}H$), 10.22 (2H, $2N_{(3)}H$ in H_2O)
IV	56-59 (25 mm merc. column)	F 20.75 (20.76)	1.19 (6H, $J^3_{H,H}$7.2 Hz, $2CH_3$), 4.10 (4H, $J^3_{H,H}$7.2 Hz, $2OCH_2$)
V	156-159	10.55 (10.63)	1.00-1.35 (6H, $J^3_{H,H}$7.0 Hz, $2CH_3$), 3.73-4.31 (4H, $J^3_{H,H}$7.0 Hz, $2OCH_2$), 7.78 (1H, $J^2_{H,H}$10.0 Hz, $C_{(6)}H$ (Het)), 11.69 (1H, $N_{(3)}H$ (Het))

New compounds I-III, V was tested on the heterotransplantates of man's glioma cancer of brain (by Bogden's under capsule-method) and Carcinosarcoma Wokera 256.

MATERIALS AND METHODS

Objects of investigations: new heterocyclic mono- and bis-adducts of 5-nitrouracile with ftorotan (1,1,1-threefluoro-2-bromo-2-chloroethane), bacterial lectine (*Bacillus polymyxa 102 KGU)*, molecular complexes of adducts which synthesized with bacterial lectine. The majority of the absolute organic solvents (benzene, dimethylformamide (DMFA), hexane, ethyl ester) employed in the present studies were distilled before use. Organic solvents were dried over anhydrous magnesium sulfate or metallic sodium. The structure of synthesized compounds has been confirmed by data of elemental

analysis. Gas-liquid chromatography carries out on Perkin Elmer chromatograph with UV-detector ("Perkin", Germany). IR spectra were recorded in a UR-20 spectrometer ("Charles Ceise Hena", Germany). The ^{1}H NMR spectra were recorded in DMSO-d6 on a 200 MHz BrakerWP-200 ("Braker", Switzerland) or Varian T-60 spectrometer ("Varian", USA). New compounds with significant antitumour action selected and investigated (Welchinska, 2003). The white imbredical mice and experimental model of tumour growth (operation and biopsy materials of man's glioma cancer of brain, heterotransplantates and Carcinosarcoma Wokera 256) were used following published procedures as Perevodchikova et al. (2005) writes. The experimental tumours used for our investigation were obtained from Bank of stammes of Oncological Centre of Russian Academy of Medical Sciences. 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 National Academy of Medical Sciences of Ukraine. The efficiency parameter [% of growth relaxation of tumour, (volume and mass)] is $\geq$ 25% in case of man's glioma cancer of brain and $\geq$ 50% in case of Carcinosarcoma Wokera 256. The results were assessed by standard methods of statistical analysis (Prozorovskiy, 1978; Sophiyena, 1979). Investigation of critical toxicity of new compounds was carrying out at Institute of Pharmacology and Toxicology of National Academy of Medical Sciences of Ukraine. Way of introduction - under skin. The lectin's preparations were obtained by treatment of *Bacillus* bacteria *(Bacillus polymyxa 102 KGU)* 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 (Podgorskiy, 1992). Molecular complexes of bis-adducts and bacterial lectins were obtained by mixing of two components at physiological solution, 1:1.

EXPERIMENTAL PART

1. Chemistry

*General procedure of the preparation of N $_{(1)}$-(1',1'-difluoro-2'-bromo-2'-chloroethyl)-5-nitrouracile (**I**), N$_{(1)}$-(2'-bromo-1'-hydroxy-2'-chloroethenyl)-5-nitrouracile (**II**).* Solution I. A mixture of potassium hydroxide (0.44 g, 0.0079 mol) and dibenzo-18-crown-6-complex (0.044 g, 0.0079 mol) in 20 ml of dry benzene was heated under reflux at 60-80°C for 15 min. The cooled solution was mixed with halothane (1.57g, 0.84 ml, 0.0079 mol) in 20 ml of dry ethyl ester. A solution I was heated under reflux at 60-80°C for 15 min. After that the cooled solution I was mixed with solution II [5-nitrouracile (1.24g, 0.0079 mol) in 40 ml of dry dimethylformamide] and then heated under reflux at 60-80°C for 3 h. The heated solution was filtered. The precipitate was washed with 30 ml of mixture of ethyl ester-hexane (1:1), dried under vacuum. The product **I** is a yellow-colored solid (48%). $C_6H_3BrClF_2N_3O_4$. γ_{max} (KBr), cm^{-1} 550-690, 1370-1390, 1550-1580, 1710, 1750.

Cooled filtrate stay per night. Remainder (product **II**) - oil which crystallized from the mix of ethyl ester-hexane (1:1). Solid which obtained dried on the air (12%). $C_6H_3BrClN_3O_5$. γ_{max} (KBr), cm^{-1} 550-690, 1370-1390, 1550-1580, 1710, 1750, 3200-3400.

*N$_{(1)}$,N$_{(1')}$-(2"-bromo-2"-chloroethenyl)-bis-(5-nitrouracile) (**III**).* The adduct was prepared according to the general procedure. The product **III** is a cream-colored solid (56,5%). $C_{10}H_4BrClN_6O_8$. γ_{max} (KBr), cm^{-1} 550-695, 1710, 1750.

*1,1-diethylcarboxy-2-treefluoromethyl-2-chloroethylene (**IV**).* A mixture of metallic sodium (6.13g, 0.268 mol) in 250 ml of methanol anhydrous, diethyl ester of malonic acid (43.0g, 40 ml, 0.268 mol) and treefluoroacetic acid (62.0g, 40 ml, 0.543 mol) was heated under reflux at 60-80°C for 6 h. To the product - glass-shape mass with white color added ethyl ester. The precipitate is white-colored solid (product A). A mixture of product A (8.0g, 0.0287 mol) in 55 ml of dry dichloroethane and phosphorus pent chloride (6.0g, 0.0287 mol) was heated with boiling for 5 h. The precipitate was filtered and washed with dichloroethane. The product **IV** is oil (80%). Boiling point: 56-59°C (25 mm of merc. column), n^{25}_{D} 1.3010. $C_9H_{10}ClF_3O_4$. γ_{max} (KBr), cm^{-1} 400, 415, 470, 560, 730, 905, 995, 1180, 1230, 1295, 1315, 1600, 1735, 2800-3000.

1,1-diethylcarboxy-2-treefluoromethyl-2-(5'-nitrouridine-$N_{(1')}$-)ethylene ***(V)***. A mixture of 5-nitrouracile (2.26g, 0.014 mol) in 50 ml of dry dimethylformamide, anhydrous tree-ethylamine (1.42g, 2.5 ml, 0.014 mol) and the product **IV** (3.8g, 0.014 mol) in 10 ml of dry DMFA was heated at 60-70°C for 6 h, heated with boiling for 10 h; filtered, $N(C_2H_5)_3$ x HC1 withdrawal. The oil yellow-colored was washed with 10 ml of hexane, 10 ml of acetone. The product **V** is a yellow-colored solid (45%). $C_{13}H_{12}F_3N_3O_8$. γ_{max} (KBr), cm^{-1} 400, 415, 470, 560, 600-800, 905, 995, 1180, 1230, 1295, 1050-1150, 1315, 1370, 1600, 1710, 1715, 3010-3080.

2. Biology

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 six. Minimum mass of mice body was 17.0±2.0 g. The age of the mice was 2-3 months. Percentage primary recovery and destruction is '0'. Method of killing was decapitation, redosage of ethyl ester. 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 (Sophina, 1979):

(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 adducts I-III, V every day. The dosage of the preparations corresponded to 1/4-1/6 of the LD_{50}. Results after 24 h of finishing of treatment were calculated. 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. The criteria of considerable is $\geq$25.0% and $\geq$50.0% of growth relaxation of the tumour. Preparation of standard was 5-flurouracile (5-FU). The express-method of definition of LD_{50} was used (Prozorovskiy, 1978). Results were calculated at alternative form after 2 weeks after the introductions. Statistical analysis carries out by (Perevodchikova, 2005). The doses of substances were from 600 to 250 mg/kg. Tonic convulsions during 1-2 h, vomiting at experimental animals were observed.

RESULTS AND DISCUSSION

The chemical composition and structure of new mono- and bis-adducts of 5-nitrouracile, toxicity and antitumour activity of new compounds and adduct of bis-derivative of 5-nitrouracile (**III**) with *Bacterial lectine 102* have been studied. It has been shown that mono- and bis-adducts of 5-nitrouracile **I-III**, *Bacterial lectine 102* are more toxicity (in 0.5-0.6 ones), than 5-FU (LD_{50} is 375 mg/kg). Meanings of its LD_{50} are from 262 mg/kg up to 185 mg/kg. Data of toxicity of these products are shown in Table 2.

Table 2. Parameters of toxicity of compounds **I-III** and *Bacterial lectine*, compared with 5-FU

№	Adduct	LD_{50}, mg/kg
1.	$N_{(1)}$-(1',1'-difluoro-2'-bromo-2'-chloroethyl)-5-nitrouracile (**I**)	185.00
2.	$N_{(1)}$-(2'-bromo-1'-hydroxy-2'-chloroethenyl)-5-nitrouracile (**II**)	241.00
3.	$N_{(1)}$,$N_{(1')}$-(2"-bromo-2"-chloroethenyl)-bis-(5-nitrouracile) (**III**)	262.00
4.	*Bacillus polymyxa 102 KGU*	248.00
5.	5-FU (control)*	375.00

Source: Author; *Prozorovskiy, V.B. et al. (1978); Sophina, Z.P. et al. (1979)

It has been established that bis-adduct of 5-nitrouracile **III** has the antitumour activity. After treatment by bis-adduct **III** the mass of heterotransplantates of man's glioma cancer reduced from 2.68±0.102 mg to 1.85±0.102 mg. It's 27.48% of growth braking of the cancer's growth (criteria of considerable is ≥25.0% of growth relaxation of the tumour). It's in 1.1 ones more than standard criteria during the treatment of glioblastome which confirmed by carry out of morphological control. A strongly antitumor effect of molecular adduct (bis-derivative **III** - Lectine 102) on Carcinosarcoma Wokera 256 tumour with percents of growth relaxation of tumor mass 79,27% (the criteria are ≥ 50%) has been registered. It's in 1.6 ones more than standard criteria during the treatment of Carcinosarcoma Wokera 256 tumour which confirmed by carry out of morphological control.

CONCLUSIONS

New mono- and bis-adducts of 5-nitrouracile, adduct of bis-derivative of 5-nitrouracile and *Lectine 102* were prepared and tested for their toxicity and antitumour activity on the heterotransplantates of man's glioma cancer of brain (operation and biopsy materials; by Bogden's under capsule-method) and on Carcinosarcoma Wokera 256 tumour. A new convenient methods for the preparation of heterocyclic mono- and bis-adducts **I-III**, **V** of 5-nitrouracile with l,l,l-trifluoro-2-bromo-2-chloroethane (halothane) or l,l-diethylcarboxy-2-chloro-2-trifluoromethylethylene are described. The reactions are catalyzed by DB-18-crown-6-complex (at the alkali medium). Investigation of the critical toxicity of compounds which synthesized shows it has a little toxicity: LD_{50} from 262 mg/kg up to 185 mg/kg. Antitumour activity of bis-derivative of 5-nitrouracile **III** and it's adduct with Lectine 102 permits to consider it as physiological active with a perspective investigation as potential antitumour drugs for treatment of man in future.

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