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DEVELOPMENT OF METHODS OF SYNTHESIS AND STUDY OF ANTITUMOR ACTIVITY OF THE SUBSTANCE – GLYCOSIL-NMM-1

РАЗРАБОТКА МЕТОДОВ СИНТЕЗА И ИЗУЧЕНИЕ ПРОТИВООПУХОЛЕВОЙ АКТИВНОСТИ СУБСТАНЦИИ – ГЛИКОЗИЛ-НММ-1

СИНТЕЗ МЕТОДДОРУН ИЗИЛДӨӨ ЖАНА ГЛИКОЗИЛ-НММ-1 КОШУЛМАСЫНЫН ШИШИК ООРУСУНА КАРШЫ АКТИВДҮҮЛҮГҮН ТЕКШЕРҮҮ

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With the purpose dilating an assortment of medical products, a synthesis and study of biologically active materials for using them in oncology practice prolongs to remain one of actual directions of modern medicine. It is known, at the same time, which only no much of synthesized drugs for medicinal administration find practice use. The majority of received compounds no find clinical use because of high toxicity, feeble aqueous solubility, nonselective action and some other by-effects. Therefore very important is represented the search of ways “upgrading” of physiologically active compounds by combination them with natural objects of natural parentage, in particular, with carbohydrates, without essential change of functions of determinant groups.

In the oncological practice are used successfully of numerous antitumor drugs, which are representing different classes of chemical compounds, which are having

different mechanisms of action, antitumor activity and also side effects.

The opening an antibiotic “strepthozotocine” and establishment of spectrum its biological activity were stimulated sharp augmentation of the works at synthesis analogs of “strepthozotocine” as well as allocating drug by microbiological which is possessing antitumor activity is a glycoside nitrozomethylurea.

The antimitotic action of derivates of an urea are connected with that, they inhibit a synthesis of deoxyribonucleic acid (DNA), oppress activity the enzyme ribonucleotide to deoxyribonucleotide, that this group of antitumoral drugs amazes of cells only in the stage S of cellular cycle.

In the present work we are prove the search and were developed new methods of a synthesis more active antitumoral compound – glycosil-NMM-1 among derivates of nitrozourea and is lead of a study its antitumor activity on the experimental animals.

The researches kinetic and catalysis of reactions of carbohydrates with participation of glycoside linkages is important not only for the theory structure and reactionary ability of carbohydrates. They represent appreciable interest and for decision of some actual problems of bioorganic chemistry as glycoside linkage is one of the important structural elements of many biologically active substances. They had been studied reactions of formation and hydrolysis of N-glycosides with amines of various natures, oxidative dehydrogenation of monosaccharides. The purpose of study all specified reactions of N-glycoside centre was establishing of mechanisms of acid-base, nucleophilic catalysis that is a few studied areas of chemistry of monosaccharides and them derivates. These researches were allowed to install some prominent features of this view of catalysis on the example of objects, which are differed with high labiality and predilection to various transformations in the conditions of catalysis by acids and bases. The data saved up in the literature about possibility using them as initial substances for synthesis of many practically important derivates of carbohydrates and polysaccharides for development of effective methods of synthesis physiologically active structures. It is necessary to note, that synthetic capabilities of this class derivates of saccharums at the present in a due measure are not opened [4].

In this connection we concentrated attention on the synthetic aspects of homogeny catalysis and development of methods of directional synthesis of a carbohydrate containing physiologically active materials. They have important value in making drugs with medical purpose, in particular, antitumor drugs, which are possessing with a high water solubility, small toxicity and selective action [1, 2].

The considering difficulties in a preparation of synthesis of derivates with specific олиго-and polysaccharide fragments is represented of expedient at the first stage of researches to pay attention to studying of chemistry and pharmacology with more simples of monosaccharide rests. These researches represent interest for decision of practically important problems of "upgrading" physiologically active substances and medicinal preparations with knows therapeutic actions. And given direction provides a development references for depression of toxicity, change of water and lipid solubility of preparations, and also the reception derivatives with selective permeability through cellular membranes.

By pharmacological examinations had been installed, that glycosilation results to sharp depression of toxicity of medicinal preparations (LD_{50} is decreased, as a rule, on the two orders). The aqueous solubility is increased simultaneously.

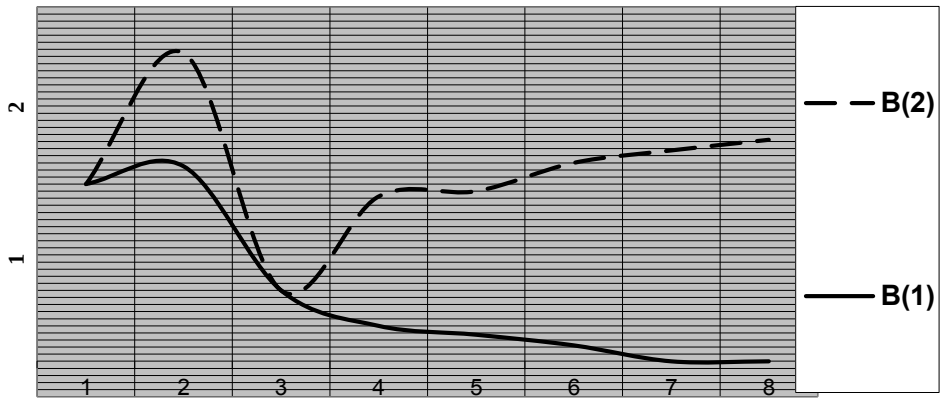
In the clinical oncology a great value has not only high antitumor activity of preparations from the group nitrozourea, but them ability to get through blood-brain barrier, that opens real opportunities for the chemotherapy of metastasizes and primary tumors of the brain. The majority of know preparations no pass through blood-brain barrier and consequently no warn diffusion of metastasizes in the brain and they practically are not effective at lesion of central nervous system.

What is the selective action, is known, that permeability of some cellular membranes of animals in relation to monosaccharides is vary, and a degree of permeability is correlated with nucleophilic reactionary ability of monosaccharides (Fig. 1) [11].

From this follows basic opportunity of adjustment processes of passage of carbohydrate preparations through cellular barriers by changing of the nature of carbohydrate carrier and also them biological activity. It attracts an attention and

Relative absorbability B (1) and relative reactivity B (2) monosaccharoses on C (1)

Fig. 1.



1- glucose; 2- galactose; 3 - laevulose; 4 -mannose; 5 - xylose; 6 - pectine sugar; 7 - ribose; 8 - lyxose;

these fact of series therapeutic properties essentially depends from the place of connection of physiologically active fragment to the carbohydrate ring. It was especially brightly shown at studying of preparations with an antitumor action.

The certain interest represents comparison of biological properties of bonds with the cyclic and acyclic carbohydrate rests. What is the ways of connection to the monosaccharide complex of physiologically active bond or the separate structural fragments, suitable for subsequent formation of the potentially active block, that here are opening rather greater opportunities.

It is perspective for practice use on our opinion is using as "joining links" of acid-steadier amide links.

In what consists value of normalization of amid communication and what is the prospects of use them in applied aspects of the chemistry carbohydrates? It is enough to specify on a wide prevalence of an urinal fragment among natural substances - derivatives of some pyramiding, nucleosides, riboflavin, theobromine, caffeine, etc., and a development direction of a synthesis nitric heteracycles including of analogues nucleosides on the basis of carbohydrate derivates of the urea.

Further have been developed intensive researches at the synthesis and studying of biological activity of derivatives of nitrozoureas in the USA, Japan and Germany,

etc. However, basic attention in these researches have been directed on studying of more complex bonds - derivatives, which are containing in the structure chlorinethyl groups, which are active fragments of most widely studied and widespread antitumor preparations [12].

From synthesized and studied in USA a little hundreds of chlorine ethyl nitrozoureas at the schema of clinical study were passed three drugs - I, 3-bis(2-chlorine ethyl)-I- nitrozourea (BCNU), carmustine, I-(2-chlorine ethyl)-3 (4-methyl)-cyclohexyl-I-nitrozourea (MeCCNU), limestone, and also above mentioned the antibiotic "strepthozotocine" (nitrozomethylurea). And in Russia clinical approbation were passed and are applied in a practice nitrozomethylurea and its carbohydrate derivate - I-methyl-3-α-L-arabinosyl-I-nitrozourea [6].

All above-stated, applied in the practice anti blast drugs are synthesized by multiphase expedient with application of deficient compounds and are concerned to difficultly accessible, expensive drugs.

In the synthesis of N-glycosyl derivates of an urea were used two basic methods of direct condensation of the urea with no protecting saccharums in a presence of mineral acids, interaction of acyl replaced N-glycosil iso-(tie)cyanides with amines or interaction of acyl replaced glycosil amines with arylisocyanides.

The method of direct condensation developed by Shari and modified by Dudkin, demands withstanding admixture the glucose and urea in a water solution in presence of the acid at 50° in during 7 days, dropped out an adduct after evaporation in a vacuum decompose by boiling in the ethanol in during three days [5, 9]. Repeated attempts of acceleration of a reaction by means of various acidic catalysts were not led to significant successes.

The second method of amineting of glycosilisocyanides and glycosilisotiecyanides was found wide application, despite on a lot stage of process [9]. The method is universal as possible to enter of urea fragment into any position of carbohydrate ring at retention of aminogroup in this position.

Many derivates of glycosilnitrozourea have been synthesized at a reaction of interaction monosaccharide and alkylamine with subsequent processing of received

glycosilnitrozes by alkylisocyanides. In a result under the action of formic acid isomerizes in thermodynamic of a stable shape of glycosylurea. Received glycosilurea nitrosates and receive derivates of glycosilnitrozourea [6, 7].

The development of works at specified directions essentially depends from development of technical comprehensible and economic expedients of introduction of a carbamide fragment in the mono- and olio saccharin's. We are developed convenient and an effective method of N-glycosilation on a basis studying of kinetic and gear of reaction of direct N-glycosilation in the requirements of a nucleophilic catalysis.

We developed simple expedients obtaining of glycosilcarbamides and addressed to the synthesis of carbohydrate derivates of nitrozoalkylureas with a purpose revealing of more active antitumor drug. In a result of search requirements for obtaining N-nitro derivates of monosaccharides with non protecting hydroxyl groups, which are providing simplicity, efficiency of a process and capability of assimilation this process in trial requirements, we were fulfill a blanket procedure for obtaining N-alkyl-N'-(β-D-glycopyranosyl)-nitrozourea in which had been combined reactions: a) interactions of monosaccharides with alkylurea in requirements of a nucleophilic catalysis with adding of arylamines and b) nitrosification of N-alkyl-glycosilureas.

As monosaccharin's were used D-glucose, D-galactose, D-kcylose, L-arabinose, L-ramnose, and as alkylureas – methyl-, ethyl-, propyl-, butyl ureas [3].

Thus, it was developed enough a simple and portable method of a synthesis of major bunch of a class nitrozoalkylurea capable to provide a flexible guidance of framework of synthesized bonds.

On this bottom we are represented perspective examinations on the antitumor activity of modified by mean of a glycosilamide link of knows medical products, in particular, nitrozocarbamides that to create of drugs higher anticancer activity.

For obtaining of many practically important carbohydrate derivates of a nitrozourea and polysaccharides, the development of effective methods of synthesis physiologically active materials are used N-glycosides. They have great importance in making of drugs for medical administration, in particular, antitumor drugs, which

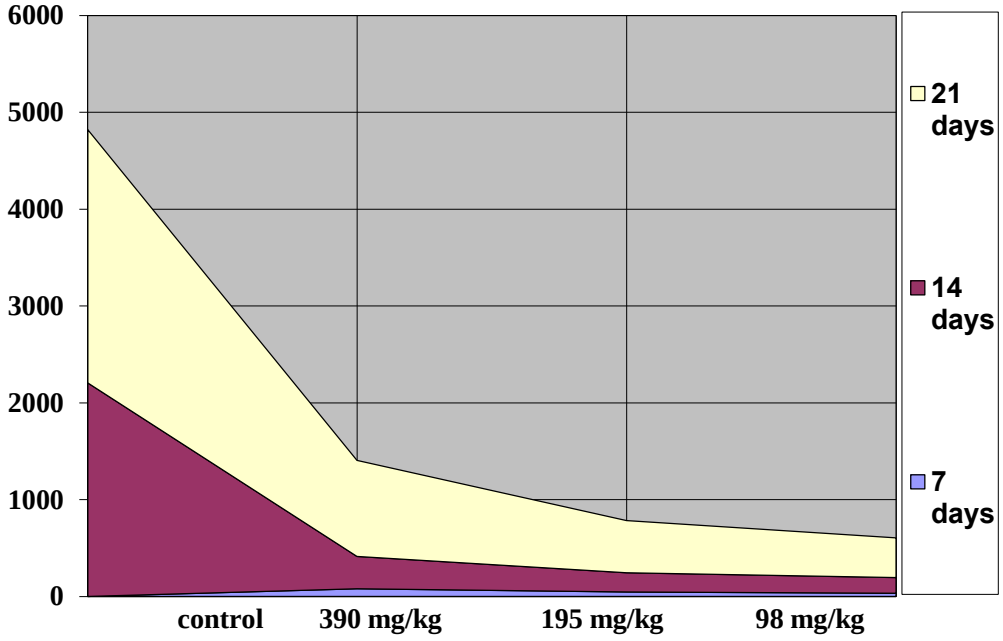
are possessing a good solubility in the water, small toxicity and selective of action.

The sharp toxicity of a glycosil-NMM-1 was defined on a white non pedigrees mice-males with a mass 18-22 g by intraperitoneal injection. The result was estimated in 24 hours after the injection of an explored substance [8].

Antitumoral properties were studied according to «Methodical references at primary selection of antitumor preparations» (1980) on the animals-tumor carriers – at a hypodermic variant of the Ascite tumor of Ehrlich by comparison of a kinetic height of tumors at both control and experimental animals and registration a share of a tumor process (Fig. 2-3) [10].

Effect of glycosil-NMM-1 at intraperitoneal injection on a mass of Ascite tumor of Ehrlich

Fig. 2



In the examinations were used linear white mice-males, on which adequately was generated of tumor process and more evenly were developed tumors. The Ascite tumor of Ehrlich at a subcutaneous version is a tumor a high degree of malignant. At this version of tumor capability the exact injection of tumor cells and fast height of pathological process does to this strain of the tumor a convenient test-object at primary selection of antitumor preparations.

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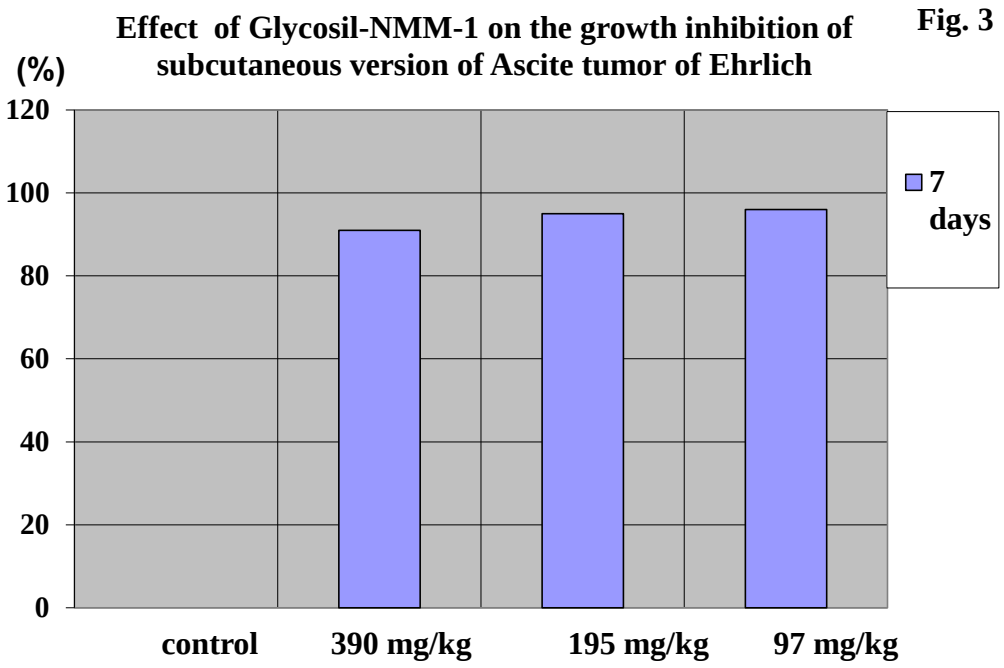

Fig. 3

tumor of Ehrlich at a subcutaneous version is a tumor a high degree of malignant. At this version of tumor capability the exact injection of tumor cells and fast height of pathological process does to this strain of a tumor a convenient test-object at primary selection of antitumor preparations.

The intertwine a tumor was conducted to 100 linear white mice-males of a mass body 18-22 g. The experimental animals were divided into 4 groups. The animals of skilled bunches received glycosil-NMM-1 by intraperitoneal in doses: 1/5, 1/10 and 1/20 mg/kg LD_{50}. To the animals of control groups was entered the isotonic solution of sodium chloride (0,9 %).

Antitumor activity at a modeled tumor was defined at changing of mass of a tumor in vitally and dynamic in 7, 14 and 21 days in the end of a course of treatment.

The study of effect of a glycosil-NMM-1 at intraperitoneal injection in doses 390, 195 and 97 mg/kg to experimental animals, on a subcutaneous version of an Ascite tumor of Ehrlich was shown reliable decrease of mass of a tumor in all skilled groups at comparison with the check. The inhibition growth of a tumor was marked in 91 to 93 % at comparison with the group which is received isotonic solution of sodium chloride.

Thus, the results of leaded tests were shown, that a new antitumoral compound - glycosyl-NMM-1, which is concerning to the carbohydrate containing derivates of a nitrozomethylurea, possesses hypotoxicity and shows expressed antitumor action on a subdermical variant of an Ascite tumor of Ehrlich, reliable reducing of mass of a tumor and braking its growth at a tumor carried animals.

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