Synthesis of Nucleosides`s Analogues and their Application as Chemotherapeutic Agents

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Abstract

Application of the chemical-enzyme approach which is a combination of chemical synthesis and enzyme reaction has allowed to develop original effective methods of synthesis both new compounds and known nucleoside antibiotics which have been inaccessible earlier. Among them preparations for the treatment of oncohaematological diseases like: Cytarabin, Leukladin (Cladribine), Fludarabel, ara-adenosine (Vidarabine), ara-guanosine and produced from the last by chemical method a new medicine for the treatment of T-cellular lymphomas – Nelarabine.

Results on the comparative study of antileukemic activity of purine nucleosides using the model of the lymphoid leukemia L1210 are presented. It is shown that the model of mouse lymphoid leukemia L1210 in vivo is not useful for results extrapolation for human if specific activity of modified nucleoside shows itself in therapy of hair-cellular leucosis (HCL), acute myeloid leukemia (AML) and especially for T-cellular leucosis.

From commercially available derivative of 2'-fluoro-arabinofuranose developed an original method for the synthesis of antitumor drug Clofarabine for treatment of acute lymphoblastic leukaemia (ALL) in pediatric patients.

Highly effective technology producing pharmaceutical substance Pemetrexed has been developed. At present new chemical-therapeutic agent Pemetrexed, which is structural analog of folic acid containing 7-deasaguanine is introduced into clinic practice. It is first agent specially developed for treatment of malignant pleural mesothelioma. It is worth to note that possibilities of the Pemetrexed's usage for treatment of other tumor diseases are not exhausted still.

It is interesting to develop new method of synthesis of modified pyrimidine nucleoside Azacytidine possesses both cytostatic action and ability to inhibit DNA methyltransferases.

Introduction

Analogs of nucleonic acids components are playing the central role among anticancer and antivirus medicines during last 40 years. It seals, firstly, with key role of DNK and RNK in different processes of cell functioning and, secondly, with big achievements in biomedical sciences and pharmaceutical technology.

Nucleonic acids play the vital role in storage and transfer of hereditary information and consequently control all processes of cell living activity from its appearing up to its destruction. Nucleosides are structural units of nucleonic acids and usually don't show any biological activity. There was an opinion that they could not be a source for chemical therapeutic matters. Later it has been supposed that some changes in carbohydrate and/or heterocyclic part of nucleosides could result in formation of compounds with interesting biological properties. First results of such approach to search of biologically active compounds gave positive results and new medicines for treatment of virus infections and for chemical therapy of cancer have been introduced into medical practice [1-2].

From 1970 in Laboratory of Nucleotides and Polynucleotides of Institute of Bioorganic chemistry NAS of Belarus complex study of chemistry, biochemistry and biotechnology of nucleonic acids components is carrying out. As a result of investigations new highly effective methods for synthesis

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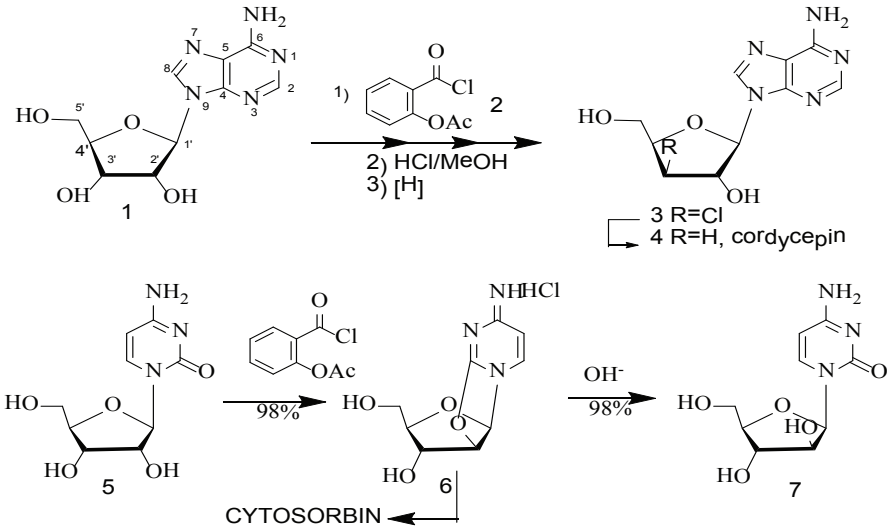
of different analogs of nucleosides, nucleotides and olygonucleotides have been proposed for application both in medicine and agriculture [3-6].

Development of new synthesis methods aiming modification of natural nucleosides is the direction in search of biologically active compounds. So, treatment of adenosine (1) with acetylsalicyloyl chloride (2) results in chlorine derivate of adenosine (3). Further reduction of the last allowed to produce nucleoside antibiotic Cordicypin (4) [3] (Scheme 1).

Transformation of cys-diolic group of cytidine (5) resulted in cyclocytidine (6) which is antileucosis medicine known as Ancytabin in spite of expected chlorine derivate. Composition of Ancytabine with modified cellulose allowed in collaborationwith RUE "Belmedpreparaty" to produce original optic liniment which is characterized with cytostatic (antiproliferate) activity.

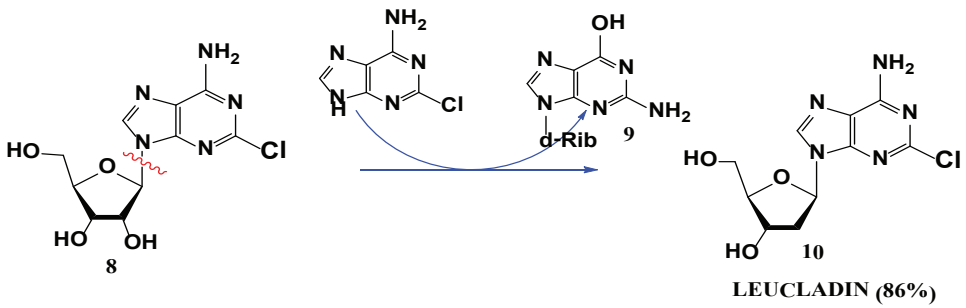
Treatment of cyclocytidine (6) with solution of alkali resulted in cycle's break and formation of Cytarabine (7). This simple scheme became the best technology to produce oncology drug Cytarabine. Based on this original technology Experimental plant of Institute of Organic synthesis (Latvia) started in 1987 to produce in industrial scale active pharmaceutical ingredient (API) and Cytarabine [3].



Scheme 1.

Chemical methods of nucleosides synthesis are steel very laborious in spite of considerable progress in this area. Microbiological methods of production applying ferments of exchange of nucleonic acids as bio catalysis attract more and more the attention of researchers.

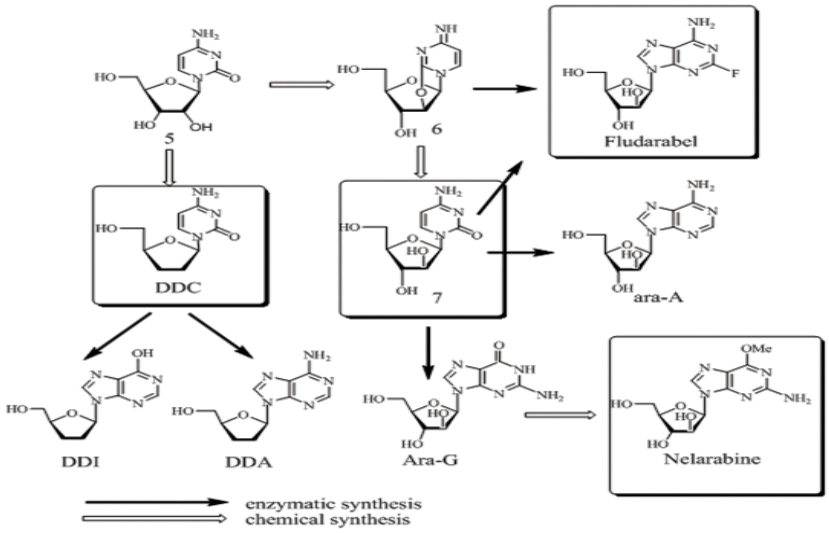
Chemical-enzymatic synthesis of antineoplastic agents of new generation Leucladin (10) based on natural 2-desoxiguanosine (9) and chemically synthesized 2-chlorinadenine (8) is shown in Scheme 2. Application of selected in Institute of Microbiology of NAS of Belarus the whole cells of *E. coli* BMT-38 as biocatalyst revealed remarkable peculiarities of this transglycosylation reaction and allowed to simplified very notably the technological process and reduce the cost of nucleoside 10.



Scheme 2.

Joint investigations carried out by Institute of Bioorganic Chemistry and Institute of Microbiology of NAS of Belarus resulted in experimentally proved highly effective chemical-enzymatic approach to produce modified nucleosides, especially if their synthesis by only chemically or microbiological synthesis is very difficult or impossible [7]. This approach originally combines methods of biotechnology and fine organic synthesis and joins advantages of chemical and biological technologies. Moreover, the whole (intact) cells of microorganisms are used as biocatalysts in spite of difficulty accessible purified ferments [8].

In Scheme 3 one can see the probability to produce different anticancer and antivirus agents applying developed approach. Combination of chemical synthesis (light arrow) and microbiological one (dark arrow) allows to produce known but difficulty accessible before nucleoside antibiotics. Among them are the compounds to treat human immunodeficiency virus (HIV) (Zamicyt – DDC, Didanosin – DDI, Didesoxiadenosine – DDA), drugs for cancer and oncology are: Cytarabine, Fludarabel, Ara-Adenosine (Vidaribin), Ara-Guanosine and obtained chemically from it a new agent for treatment of T-cell lymphoma Nelarabine.



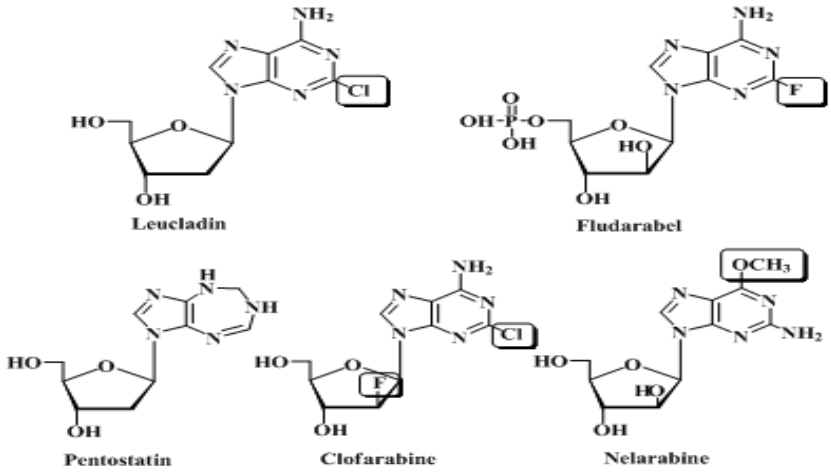
Scheme 3.

At present combined chemical therapy allows maximally acts on leucosis process on any stage of blastogenesis: induction of remission during first attack, supporting therapy during remission, chemical therapy of recidivous or blast crisis. Big progress in therapy of leucosis during last 20 years has achieved due to usage of less “aggressive” cytostatics. Analogs of purine nucleosides such as Leukladin, Pentostatin, Fludarabel, Nelarabine play keep the central place (Scheme 4).

Purine analogs have a lot of in common: their chemical structures are similar (Scheme 4), likely natural deoxi- and ribonucleosides they penetrate through cell membrane with help of effective transport system and are sequentially phosphorylate by intracellular kinases with formation of 5’-mono-, -di- and/or triphosphates. Last are in charge of inhibition of virus infection or proliferation of cancer cells. Natural nucleosides, deoxiadenosine or adenosine, rather fast deaminated in cell by adenosine deaminase into non-active inosine. Input of chlorine atom into second position of purin heterocycle of deoxiadenosine has solved the problem of sustainability of Cladribine and Clofarabine to action from adenosine deaminase. All three phosphorylated forms of nucleosides rather fast accumulate within the cell as activity level of deoxicytidine kinase considerably higher than level of 5’-nucleotidase. It is worth to note that differences in activity level of mentioned ferments in lymphocytes determine specific action of Cladribine and Clofarabine on this type of cells.

Mechanism of influence of fludarabine phosphate is rather similar with influence of Cladribine. Input of fluorine atom in second position resulted to increase of preparation stability against the influence of adenosine deaminase. Fludarabine phosphate is rapidly dephosphorylated to 2-fluoro-ara-A and then phosphorylated intracellularly by deoxycytidine kinase to the active triphosphate, 2-fluoro-ara-ATP [9].

Preparation Nelarabine is depo-form of arabinofuranosylguanine (Ara-G), which is due to low solubility did not fine wide usage in clinical practice. Inside the cell Nelarabine splits up to active Ara-G under the influence of adenosine deaminase. Mechanism of Nelarabin's influence is rather similar to influence of mentioned above preparations but their targets are different [10].



Scheme 4.

Comparative study of antileukemia activity of purine analogs on leukemia model L1210 keeping on mouse DBA_2 and re-inoculated them with hybrids CD_2F_1 is presented in Table 1 [11, 12].

Table 1

Antileukemia activity of purine nucleosides on model of lymphoid leukemia L1210 (10^6 cell/mouse intraperitoneally) for mouse-hybrids $F_1(DBA_2 \times C57Bl/6)$

Preparation	Preparation dose and "treatment" scheme	Duration of mouse survival (days), M ± m	T/C (E/C×100%), p
Control	P-p NaCl 0.45%	7.1±1.57	100
Fludarabel	300/48×4	18.0±2.53	207; <0.001
Leicladin	30/48×4	12.7±1.37	146; <0.001
Nelarabine	450/48×4	7.6±0.52	107; >0.05

Fludarabel in dose (300 mg/kg i/v, standard scheme) which is adequate to therapeutic dose for human provides the most high antileukemic effect on model of mouse lymphoid leukemia. Quantitative criteria of effectiveness (T/C) of Fludarabel in direct parallel comparison with two other purine nucleosides is 207% (Table 1). It is important to note that the loss of body's mass was very small for experimental mouse as well as for control group. Moreover, the value of ratio of mean life duration experimental and control mouse (207%, $p<0.001$) is higher in 2.4 times (E/C≤85%) compared to antileukemic preparation shown toxic cumulative effect. So, pre-clinic data obtained in chemical therapeutic experiment characterized Fludarabel as anti-neoplastic preparation with the absence of toxic cumulative effect. This generation of re-inoculated tumor is adequate and informative model for pre-clinic test and selection of preparations influence selectively on flow of B-cell chronic lymphoid leukemia and non-Hodgkin`s lymphoma. Leucladin has notably lower antileukemic effectiveness compared to Fludarabel for the model of mouse lymphoid leukemia L1210.

Leucladin inoculated in dose (30 mg/kg i/v, standard scheme), which is adequate to clinic dose for human, shows antileukemia effectiveness for criteria of longer life duration of "leucosis" mouse which is equal to 146%. The increase of antileukemia effectiveness proportionally to dose-dependant one has not been observed for higher dose of Leucladin. So, direct parallel comparison reveals that the ability of Leucladin to increase the life duration of "leucosis" mouse lower at least 2 times compared to Fludarabel. It is shown that the model of mouse lymphocytic leukemia L1210 in vivo is low valuable for prognosis and extrapolation of results for human if specific activity of modified nucleoside reveals itself in treatment of patients with Hairy Cell Leukemia and acute myeloid leukemia [11, 12]. Forein researchers claimed as well about non-adequate of model of mouse lymphocytic leukemia L1210 for pre-clinic tests of Cladribine.

In experiments *in vivo* with implanted cells of mouse lymphocytic leukemia L1210 Nelarabine has not shown statistically valuable antileukemia effect even for maximum acceptable dose (taking into account ratio between dose for animals and human) (450-900 mg/kg i/v, standard scheme) (Table 1). Perdition of experimental mouse took place faster compared to control animals when the dose of Nelarabine was increased as much as twice.

Testing results of direct cytotoxicity of purine nucleosides *in vitro* on culture of stable line of human leucosis MOLT-4 are shown in Table 2.

Table 2
Cytotoxic effect of purine nucleosides *in vitro* on cells of human T-lymphocytic leukemia

Nucleosides	IC_{50}, μM, inhibiting growth of cells of leucosis MOLT-4
Nelarabine	0.97±0.15
Fludarabel	0.91±0.1*
Leukladin	1050.0±210.0

* only IC_{30} is determined

It is clear that Nelarabine shows high authentic inhibit effect in culture of T-lymphoblast cells of human leucosis (IC_{50} = 0.97±0.15 μM). Nelarabine investigated is identical to foreign drug Arranon (Nelarabine) concerning cytotoxic activity. Fludarabel has shown rather moderate cytotoxic effect in culture of T-lymphoblast cells of human leucosis and when the inhibition of cell growth reaches the level of 30% there is not a proportional dose-dependant increase of this effect.

Cytotoxic effect of Leukladine determined in vitro by means of direct calculation of blast cells in culture of human T-lymphocytic leukemia MOLT-4 reveals that Leukladine inhibits the growth of blast cells higher up to 50% compared to intact control for concentration of agent equal to 1050.0±210.0 μM. So, Nelarabine exceeds very impressively one of the most active antileukemia drug Leukladin of its selective influence on cells of T-lymphoblast human leucosis. In spite of considerable achievements of biotechnological approaches for nucleosides synthesis sometimes ferments show their selectivity and can not accept modified sugars or heterocyclic. That is why in Laboratory of Nucleotides and Polynucleotides's Chemistry of Institute of Bioorganic chemistry of NAS of Belarus investigations devoted to the development of original technologies of fine organic synthesis of biologically valuable agents are carried out. It was mentioned above that input of fluorine atom into nucleoside molecule results in the increase of its chemical and metabolic stability, change of biological activity of nucleosides and, sometimes, in improvement of antitumor and antivirus activity. Fluoronucleosides have unusual stereo-electronic properties and that leads to limitation of conformational mobility of molecule and only one preferable conformation of pyranose cycle is typical for such nucleosides in solution [13, 14]. The position of fluorine atom in carbohydrate fragment of nucleoside is the main factor for appearance of potentially biological effect and possible medical application of analogues.

Among different fluorination of purine nucleosides the analogues 2'-fluoro-2'-deoxy-ara-adenosine with chlorine atoms in second position of heterocyclic base known as Clofarabine has high activity against some tumor cell lines and is used in medical practice as effective therapeutic agent of new generation during treatment of children with acute leukemia. Original preparative method for synthesis of pharmaceutical substance Clofarabie out of accessible derivates of 2'-fluoro-arabinose has been developed and patented in Republic of Belarus [15]. In scientific-production center (SPC) "ChemPharmSintez" the organization of manufacturing of pharmaceutical substance and anticancer drug Clofarabine has been started.

At present new chemical-therapeutic agent Pemetrexed, which is structural analog of folic acid containing 7-deasaguanine is introduced into clinic practice. Pemetrexed is transported into cells by both the reduced folate carrier and membrane folate binding protein transport systems. Once in the cell, Pemetrexed is rapidly and efficiently converted to polyglutamate forms by the enzyme folylpolyglutamate synthetase. The polyglutamate forms are retained in cells and are even more potent inhibitors of thymidylate synthase and glycinamide ribonucleotide formyltransferase. Polyglutamated metabolites have an increased intracellular half-life resulting in prolonged drug action in malignant cells. Agent was registered in 2004 for treatment of spread non-small cell lung cancer (NSLC) as second-line for patients after chemotherapy. In 2008 it started to use as initial treatment in combination with cisplatin for primary patients with NSLC [16]. Pemetrexed is effective for treatment of malignant pleural mesothelioma, in combination with cisplatin [17]. Moreover, it is first agent specially developed for treatment of malignant pleural mesothelioma. Original highly effective technology producing pharmaceutical substance Pemetrexed has been developed. It is worth to note that possibilities of the Pemetrexed's usage for treatment of other tumor diseases are not exhausted still.

It is interesting to develop new method of synthesis of modified pyrimidine nucleoside Azacytidine possesses both cytostatic action and ability to

inhibit DNA methyltransferases and as a result to renew expression of gens which are abnormally depressed by hypomethylation. DNA hypomethylation of aberrantly methylated genes involved in normal cell cycle regulation, differentiation and death pathways may result in gene re-expression and restoration of cancer-suppressing functions to cancer cells.

First of all, agent Azacytidine is useful to treat myelodysplastic syndromes (MDS). Azacytidine can be applied for different types of MDS; its effectiveness to treat patients with intermediate-2 and high-risk. It is worth to note that application of this agent prevents the development of acute myeloid leukaemia among patients with MDS and improves the live quality of patients according to European Organization on Cancer Investigation and Treatment [18].

Conclusion

Taking into account the importance for native health care activity on development high effective oncology drugs the low-tonnage production of high effective pharmaceutical substances and medicinal matters has been organized on the base of developed technologies. SPC "ChemPharmSintez" at Institute of Bioorganic Chemistry, National Academy of Sciences of Belarus runs activity in area of fine organic chemistry and has unique industrial facilities allowing organization of complete cycle: from laboratory development of molecules to production and commercialization of ready preparations which can replace expensive foreign analogs.

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