DEVELOPMENTS IN THE RESEARCH OF NEW ANTITUMOR AGENTS* (REVIEW)

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UDC 616.006.04:615.277.3(047)

The following review discusses major directions in the research and preparation of various classes of advanced antitumor agents: synthetic materials which work by an alkylating mechanism (derivatives of di(2-chloroethyl)amine, ethylenimine, alkylnitrosourea, dimethyltriazines); platinum derivatives; dispiropiperazines; alkylating agents derived from polyols; various antimetabolites; as well as compounds derived from natural products, such as anthracycline antibiotics, bleomycin, actinomycin, mitomycin, derivatives of aureolic acid, etc. Also discussed are the use of enzymes to destroy substances needed for tumor growth; new plant alkaloids which inhibit mitosis (Vinca alkaloids, Podophyllin toxins, colchamine, etc.); and immunomodulating agents.

The purpose of this paper is to review the chemistry of recent developments in cancer chemotherapy, to outline new directions in interdisciplinary antitumor research involving biochemistry, organic chemistry, and medicine, and to compare research trends in the USSR vs. capitalistic countries, and the US in particular. The different classes of antitumor agents have been discussed in reviews and monographs [1-5].

At the present time oncologists and chemotherapists have at their disposal a wide range of medicinal agents to cure malignant tumors in man or, at least, to retard their growth. In our country 60 medicinal compounds are licensed for use in antitumor treatment; of these, 18 are hormonal. The list of authorized drugs includes 26 original and 21 imported compounds, as well as 13 reproduced compounds. In reality, doctors routinely use a smaller number of drugs, since several of the licensed materials have not been put into production. The US currently produces 32 antitumor agents, not including hormones [6]. In spite of these licensed materials, the need for a continued search for new compounds displaying antitumor activity is urgent, since many of the current drugs are insufficiently effective, especially against the most rapidly spreading forms of malignant tumors, which affect the greatest number of people (cancer of the gastrointestinal tract, breast cancer, lung cancer, tumors of the female reproductive organs, melanoma, and others); in addition, the current drugs are highly toxic. Furthermore, although tumor cells may show good initial response to chemotherapy, resistance to the drugs may develop.

The search for new antitumor agents is a difficult, expensive, and lengthy process. For example, since 1955 the National Cancer Institute of the United States (NCI USA) has studied experimentally the antitumor activity of 370,000 compounds in people; at the present time, 10,000 compounds are under investigation per annum. Of these, only 5-8 new materials will advance to clinical trials, and, of course, not every new drug will be effective in clinical practice. In the US, approximately 14 years elapse from the time of discovery of antitumor activity to the production of a material and, finally, to its practical application. These facts demonstrate why it is so important to continue to direct future effort toward the preparation of truly effective or novel antitumor agents, which differ significantly from those already in use.

The discovery of new drugs is accomplished both by directed synthesis (rational design), where the material is synthesized for specific reasons, and by random screening of substances. Random screening, particularly of natural product derivatives, is very interesting, since it may lead, albeit infrequently, to the discovery of materials displaying new modes of activity,

*Dedicated to the memory of S. A. Giller, researcher and leader blessed with an unusually keen sense for new challenges.

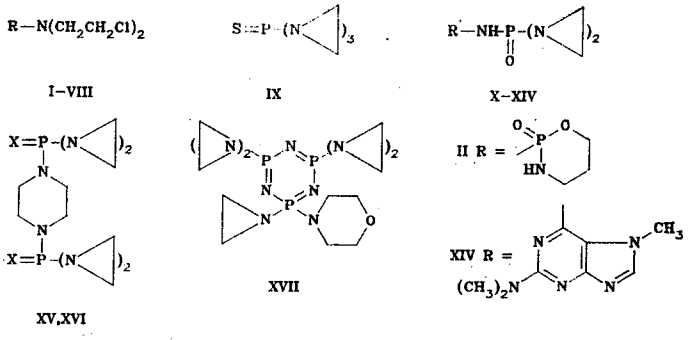
Lensovet Leningrad Technological Institute, Leningrad. Translated from Khimiya Geterotsiklicheskikh Soedinenii, No. 1, pp. 18-31, January, 1985. Original article submitted April 27, 1984.

0009-3122/85/2101-0013$09.50

which, in turn, gives new direction to the research efforts of chemists and adds new dimensions to cancer chemotherapy. At the present time rational design is focused on the synthesis of improved analogs of current drugs, i.e., second generation drugs. This area of research includes the synthesis of specific enzyme inhibitors based on a knowledge of both the mechanism of action of the enzymes and of the structure of the transition state (of enzyme activity).

The chemistry of compounds exerting their effects via alkylation represents the most highly investigated field in the research of antitumor agents [1-3, 5, 7, 8]. Substances of this type are able to alkylate the nucleic acid components of cells, which perturb their ability to function. The very first antitumor agents, namely, derivatives of di(2-chloroethyl)-amine (nitrogen mustard), belong to this class of compounds.

The first report of the therapeutic effect of di(2-chloroethyl)amine (embichin, dimitan [chlormethine]) (I) appeared in 1946 [9]; this drug is still used today in combination with other drugs in the treatment of tumors. The di(2-chloroethyl)amine residue induces the inter- and intramolecular cross-linking of DNA chains, which of course, are more accessible in rapidly dividing cells undergoing replication and transcription than in slowly dividing, quiescent cells.



I R=CH_3; III R=$C_6H_4[CH_2CH(NH_2)COOH]$-*p* (DL); IV R=$C_6H_4[CH_2CH(NH_2)COOH]$-*p* (L); V R=4-methyl-2,6-dioxo-5-pyrimidinyl; VI R=2,6-dioxo-5-pyrimidinyl; VII R=$C_6H_4[(CH_2)_3COOH]$-*p*; VIII R=$C_6H_4[CH_2CONHCH(CH_2C_6H_5)COOH]$-*p* (DL); X R=$C_6H_5CO$; XI R=4-$FC_6H_4CO$; XII R=2,5-$I_2C_6H_3CO$; XIII R=$C_6H_{11}$; XV X=O; XVI X=S

Among the alkylating agents the most important and widely used drug is cyclophosphane (cyclophosphamide, endoxan, cytoxan), RS-N,N-di(2-chloroethyl)tetrahydro-2H-1,3,2-oxazaphosphorus-2-amine-2-oxide (II). This compound is readily available and relatively inexpensive and thus plays a very important role in contemporary cancer chemotherapy. An essential element of the biological activity of cyclophosphane is its bioactivation (metabolism) in the liver, which involves initial oxidation at position 4 by means of microsomal oxidases, followed by decomposition to give acrolein and a phosphorus derivative of a nitrogen mustard, which in turn is responsible for the biological activity of the drug [1]. In the absence of nonspecific oxidases, for example in tissue cultures, cyclophosphane does not exert any biological activity. As expected, monochloroethylamines are not active, since they cannot cross-link the biopolymer molecules.

A large number of diverse derivatives containing the di(2-chloroethyl)amino moiety have been synthesized and studied. The preparation of alkylating metabolites has emerged as the most important direction of research in this area. In 1958 S. A. Larionov in the Soviet Union proposed using alkylating substances coupled with biologically active molecules. The 4-di(2-chloroethyl)amino derivative of DL-phenylalanine was prepared (sarcolysine, III); in the US the L-isomer was used (melphalan, IV). In the series of alkylating agents coupled with nucleotide bases two drugs are particularly worthy of mention: the Soviet drug dopan (5-di(2-chloroethyl)amino-6-methyluracil, V), and the American nordopan (uracil mustard, VI) [1]. It was recently demonstrated that the biological activity of melphalan in cells involves a protein carrier and not phenylalanine, as might be expected, but rather leucine [10]; embichin involves the participation of choline [11]. In this way the distinction between nonspecific alkylating agents and alkylating agent metabolites appears to be somewhat arbitrary. The study of the mechanism of action of alkylating derivatives of biological compounds has shown that the cellular transport of these drugs involves participation by specific carriers and that the delivery of the alkylating segment to the target molecule is therefore more accurate.

This conclusion is verified by the observation that alkylating agents coupled with L-amino acids are more active than D-amino acid derivatives [1].

Another example of a di(2-chloroethyl)amine derivative with practical significance is chlorambucil, 4-di(2-chloroethyl)aminophenylbutyric acid (chlorbutin, leuceran), VII. In the USSR (under the guidance of L. F. Larionov and I. L. Knunyants) a large number of sarcolysine derivatives and other alkylating derivatives of amino acids have been synthesized and studied, for example, dipeptides such as esters of N-acetylsarcolysyl-DL-valine (asalin), N-acetylsarcolysyl-DL-leucine (asalei), N-acetylsarcolysyl-DL-methionine (asamet), and others [2, 3]. The major disadvantage of these compounds is that they exist as mixtures of diastereomers which differ greatly in their biological activity; as a result, even the ratio of isomers in the drugs, frequently standardized, is not sufficiently precise. These materials have not entered into standard practice. A large number of derivatives of 4-di(2-chloroethyl)aminophenylacetic acid and amino acids were also prepared, for example, lofenal (4-di(2-chloroethyl)aminophenylacetyl-DL-phenylalanine, VIII). One should also mention fentirin, DL-4-[di(2-chloroethyl)aminophenylhydroxyl]phenylalanine [2, 3], as well as many others.

In the USSR and elsewhere various esters of 4-di(2-chloroethyl)aminophenyl substituted acetic and butyric acids with steroid hormones, for instance androgen and estrogens, have been prepared; many exhibit activity against hormone-dependent tumors [1, 3]. It is unclear whether the alkylating activity of these molecules results from attack by the hormonal residue on the hormone receptor, or whether a complex of the hormonal residue and receptor is transported to the nucleus, where it induces alkylation of the nucleic acids and chromatin.

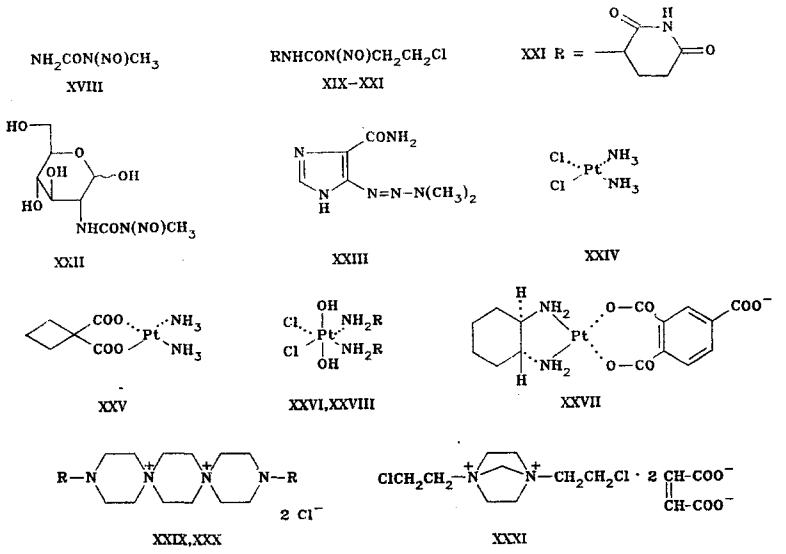
A second widely studied type of antitumor agent encompasses those containing the alkylating ethylenimine residue, notably those combined with phosphoric acid. The most important compound in this series is the triethylenimide of thiophosphoric acid (thiophosphamide, thio-TEPA, IX). In terms of frequency of use this is the second most important alkylating agent, after cyclophosphane. Other members of this class of compounds include N-benzoyldiethylenimidophosphamide (benzotef, X), its fluoro- (XI) and diiodo- (XII) derivatives (fluorobenzotef and diiodobenzotef), diethylenimido-N-cyclohexylphosphamide (hexaphosphamide, XIII), and corresponding purine (Fopurin,* XIV) and piperazine (dipin, XV, and thiodipin, XVI) derivatives, as well as the drug fotrin (XVII). Alkylating derivatives of various other compounds, particularly heterocycles, continue to be extensively studied in the USSR.

The mechanism of action of the above two types of alkylating agents is very similar, and very often tumors exhibit identical responses to all types of dichloroethylamine and ethylenimine deirvatives, i.e., a one-dimensional toxic effect. Because of this, the NCI USA does not presently accept for use any new compounds belonging to the general class of alkylating agents, not only mustards and ethylenimines, but also aklylnitrosoureas, dimethyltriazines, and epoxides [6]. In the USSR di(2-chloroethyl)amines and ethylenimines dominate the field of medically prescribed drugs (23 of 60), and the question of whether to introduce new modifications to the arsenal of antitumor drugs remains open. Regardless, the chemistry of alkylating agents continues as a major target in the research effort in the search for new antitumor agents in the USSR. In fact, dichloroethylamines and ethylenimines constitute more than half of the new synthetic antitumor agents planned for introduction during the 12th five-year period.

Other types of alkylating agents, such as nitrosoalkylureas, dimethyltriazines, and platinum derivatives, have been less extensively studied in the USSR, although the application of methylnitrosourea (MNU, XVIII) antitumor derivatives has recently begun. MNU was the first drug of this class of compounds; its antitumor activity was originally proposed by N. M. Emanuel' in 1965, based on its high mutagenicity, and the compound was prepared by I. A. Rappaport [12, 13]. In the USA in 1966-1967 various antitumor derivatives of 1-(2-chloroethyl)-1-nitrosourea with different substituents in position 3 were prepared; these include BCNU (carmustine, XIX) and CCNU (lomustine, XX). Decomposition of MNU leads to the formation of methyldiazonium hydroxide and then the methyl cation, whereas decomposition of chloroethylnitrosourea leads to the $(ClCH_2CH_2)^+$ and $(CH{=}CH)^+$ cations, which in turn alkylate the same target molecules discussed above for the other alkylating agents. In addition, the alkylisocyanates which are produced as byproducts undergo carbamoylation with cellular target molecules.

In the case of BCNU, decomposition of chloroethylisocyanate gives chloroethylamine, which can also alkylate DNA and proteins. 2-Chloroethylnitrosoureas, in contrast to methylnitrosourea, also cause DNA chains and histones to cross-link, although the biological effects

*See note p. 23.



XIX, XXX R=CH_2CH_2Cl; XX R=C_6H_{11}; XXVI R=$CH(CH_3)_2$; XXVIII R=H; XXIX R=$CH_2CH(OH)CH_2Cl$

of the two classes of nitrosoureas are similar. The role of carbamoylation in the mechanism of biological activity of the alkylnitrosoureas is unclear. It would therefore be of interest to study alkylnitrosourea derivatives capable of undergoing intramolecular carbamoylation, which cannot undergo carbamoylation with cellular targets; examples of this type of compound are 1-(1-glycosyl)-3-methyl-3-nitrosoureas [14]. Of special interest in this regard is the use of 1,1-disubstituted 3-chloroethyl- or 3-methylnitrosoureas, which are relatively stable and lipophilic compounds [15]. A large number of derivatives of N-methyl- and N-(2-chloroethyl)-N-nitrosourea as well as other biologically important compounds and heterocycles of various types have been prepared. The important properties of BCNU, CCNU, and PCNU (XXI) are their ability to penetrate the blood–brain barrier and their effectiveness against certain types of brain tumors. The relationship between the lipophilic character of this class of compounds and their biological activity is being investigated [16]. The antibiotic streptozotocin (XXII) has also found clinical application. A major complication in the study of alkylnitrosourea derivatives is their carcinogenicity, which has been demonstrated in experiments with animals. Their carcinogenicity may be attributed to their ability to alkylate nucleic acid precursors; in this way their carcinogenic effect depends upon the selectivity of various DNA and RNA polymerases to accept, or not accept, analogs in the biopolymer [17].

Various aromatic derivatives containing the dimethyltriazine group also display alkylating activity. The compound 4-(dimethyltriazino)-5-carbamoylimidazole (dacarbazin, DIK, DTIK, XXIII) has been used clinically. This compound undergoes demethylation upon reaction with NADP-dependent oxidases in organisms; the monomethyltriazine thus formed decomposes spontaneously to give diazomethane or a methyl cation and nitrogen [1, 18].

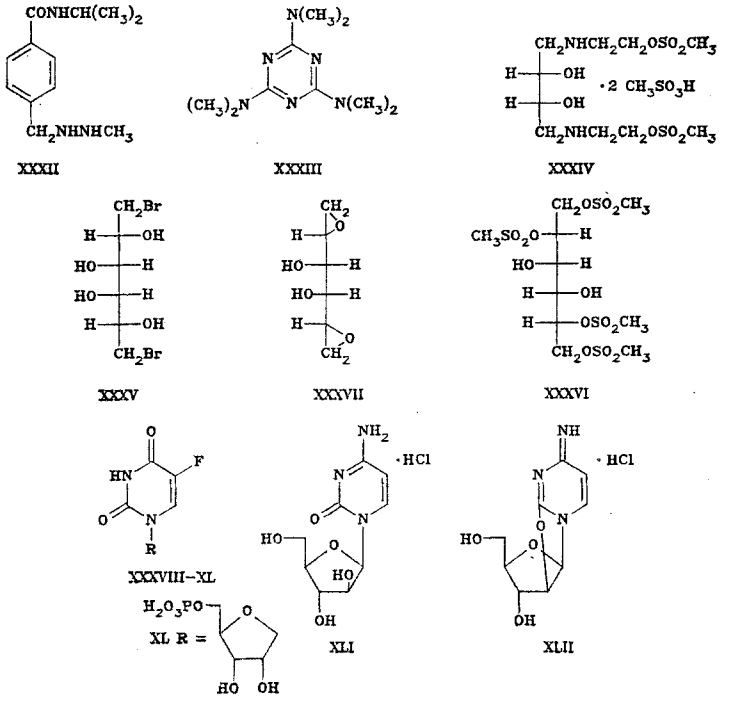
A major breakthrough in cancer chemotherapy in the past 10 years involved the introduction of platinum antitumor agents [19]. The first compound of this type, cis-diaminodichloroplatinum (platidiam,* cisplatin, DDP, XXIV) is widely used in various areas of tumor therapy. A large number of cisplatin analogs (the trans isomers are not active) were prepared in an attempt to obtain a more active, less toxic derivative (these platinum compounds are toxic to the kidney). Progress in the clinical use of this drug has contributed to the development of therapeutic methods which do not disturb kidney function. In the past several years the NCI USA has studied more than 1400 platinum derivatives; the screening of these compounds continues at the present time, although the synthesis of new analogs is no longer funded [6]. As in the case of the other classical alkylating agents, the DNA components of the tumor cells appear to be the targets of this series of compounds.

One should also mention the following interesting platinum derivatives: carboplatin* (CBDCA, (XXV); CHIP (XXVI); DACH (XXVII)). A number of drugs belonging to this class have been produced in the USSR, for instance, oxoplatin (XXVIII). The original Soviet drug Platin* is licensed for clinical use [3].

*See note p. 23.

Dispiropiperazines, prepared in the USSR, constitute a special class of drugs; members of this series include prospidium chloride, the dichloride of N,N"'-di(3-chloro-3-hydroxypropyl)-N',N"-dispirotripiperazine (XXIX); spirazidin, the dichloride of N,N'"-di(chloroethyl)-N',N"-dispirotripiperazine (XXX); and spirobromin,* the dichloride of N,N"-di(2-bromopropionyl)-N',N"-dispirotripiperazine. The mechanism of action of these compounds differs from that of the classical alkylating agents; their effect on membranes is very valuable [3]. The American derivative diazobicycloheptyl maleate (NSC 262666, XXXI) is probably analogous to these types of compounds [3].

Procarbazine [hydrochloride] (natulan), the hydrochloride of the N-isopropyl amide of p-(2-methylhydrazinomethyl)benzoic acid (XXXII), probably also belongs to this class of alkylating agents. Biotransformation of this molecule produces methylhydrazine, which gives a methyl radical upon reaction with oxygen [5, 16]. The antitumor agent hexamethylmelamine (XXXIII) also appears to be a methyl donor. Metabolism of this material gives pentamethylmelamine, which also exhibits antitumor properties [2, 3].



XXXVIII R=H; XXXIX R=tetrahydro-2-furyl

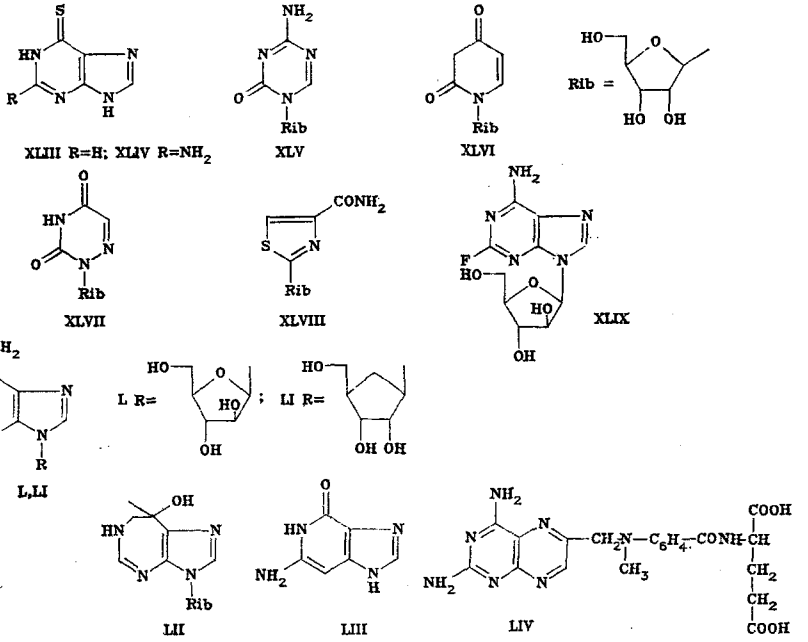
Alkylating derivatives of polyols are widely used in the Hungarian People's Republic; examples include nitrosulfan, 1,4-di(2-methylsulfonylhydroxyethylamino)-1,4-dideoxy-meso-erythritol dimethanesulfonate (XXXIV); mitolactol (dibromodulcitol), 1,6-dibromo-1,6-dideoxygalactitol (XXXV); Tsitostop,* 1,2,5,6-tetra-O-methanesulfonylhydroxymannitol (XXXVI); and DAG, 1,2,5,6-dianhydrogalactitol (XXXVII) [3].

Another important class of antitumor agents is composed of antimetabolites of nuclear metabolism. These compounds inhibit the biosynthesis of nucleic acid precursors, and also, by coupling with DNA or RNA, they can induce cleavage of the biopolymer chains and disturb their ability to function. Currently two pyrimidine antimetabolites are used clinically in the USSR; these are 5-fluorouracil (5-FU, XXXVIII) and Ftorafur† [1-2(tetrahydrofuryl)-5-fluorouracil, XXXIX], which contains the relatively nontoxic depo-form of 5-FU. Ftorafur was discovered by S. A. Giller and co-workers. This original Soviet drug is widely used not only in the USSR, but also in various other countries [20]. The hydrochloride of 1-β-D-arabinofuranosylcytosine (cytarabin, cytosar, Ara-C, XLI) and its 2,2'-anhydro-derivative (cyclocytidine, XLII) are undergoing clinical examination. The purine antimetabolites 6-mercaptopurine (XLIII) and 6-thioguanine (XLIV) are used in the treatment of leucoses.

*See note p. 23.

†Flurfucil (WHO) — Translator.

An important characteristic of these nucleic acid analogs, both the heterocyclic bases and the nucleosides, is that in order to exert a biological effect they are first converted in the cells to the corresponding nucleotides (for example, 5-fluorouracil gives the 5'-phosphate of 5-fluorouridine), and these, in turn, generate other di- or triphosphates, or deoxynucleotides. The biological activity of these analogs of nucleic acid bases and nucleosides depends upon the course of bioactivation or metabolism; if the heterocycle is capable of reacting with phosphoribosyltransferase to give a nucleotide, it will exert a biological effect. On the other hand, if the nucleotide is formed from a nucleoside which has been modified by a kinase, then it is necessary for the chemist to preform the nucleoside (or deoxynucleoside) from the heterocyclic base in order to observe biological activity [4]. The synthesis of nucleotide analogs does not offer any special advantage, because the phosphates easily permeate the cell membranes, where they are dephosphorylated. As a result, the cell is sensitized to the action of the drug, and the cellular kinases do not phosphorylate the analog (or the phosphoribosyltransferase does not phosphoribosylate it). The cell has thus developed a resistance to the exogenous nucleotide which was dephosphorylated at the cell membrane.



At the present time, a large number of antimetabolites are known and under evaluation at various stages of preclinical and clinical trials, but most have not yet been approved for general application. 9-β-D-Arabinofuranosyladenine (ara-A, L) display both antitumor and antiviral activity; it is rapidly deaminated in the cell to give 9-β-D-arabinofuranosyloxanthrene, which does not possess antitumor activity. Adenosinedeamine inhibitors, such as pentastatin* (LII) or 2'-deoxypentastatin, not only enhance the activity of ara-A, but also exert an antitumor effect on in its absence; these compounds allow adenosine to accumulate in the cell, and large concentrations of adenosine, in turn, inhibit the biosynthesis of nucleic acids [4].

5-Azacytidine (XLV), 3-deazaguanine (LIII), 5-deazauridine, and many other cytotoxic analogs are also being studied. As seen above, compounds in which the heterocyclic ring in the nucleosides has been modified (either as a result of reactions of the natural heterocycles themselves, or via the addition of substituents to the rings of the naturally occurring purines and pyrimidines) also display biological activity. Compounds in which the ribosyl or deoxyribosyl portion of the 1-β-D-arabinofuranoside has been changed, for instance by replacement with a substituted cyclopentane ring, as in the case of the antibiotic aristeromycin (LI), may also be biologically active. It is worth noting that in the USSR, in contrast to the situation with alkylating agents, the inventory of active antimetabolites which are ready for inclusion in the registry of antitumor drugs is not large. The complexity of research in this field is due not only to problems in the synthesis of nucleosides, but also the fact that the research occurs at the interface of synthetic chemistry, biochemistry, and molecular biology. In order for antimetabolites to progress successfully toward general medical ap-

*See note p. 23.

plications, it will be necessary not only to exploit the already available resources mentioned above, but also to study and ascertain the mechanisms of their bioactivation and catabolism, the structures of the active metabolites, and their enzyme targets within the cell.

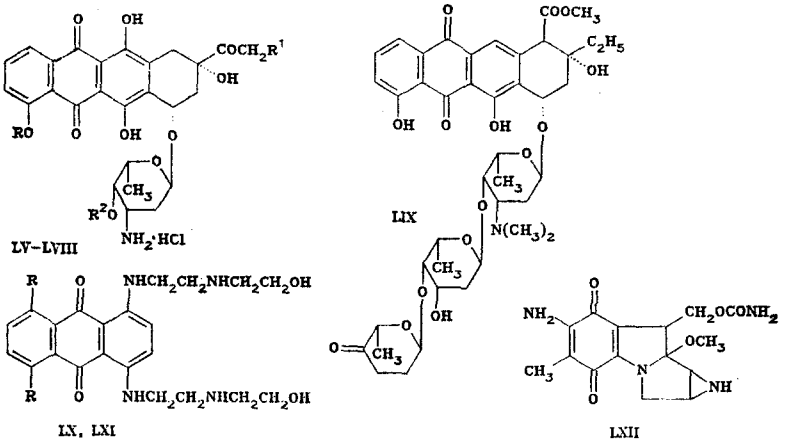
These developments would allow one not only to optimize the therapeutic effect of a drug but also to enhance the activity of the drug on the tumor cell via the addition of a secondary drug as modifier. For example, the addition of thymidine protects 5-fluorouracil from catabolic processes and enhances its biological effectiveness, whereas allopyrinol (4-hydroxypyrazolo[3,4-d]pyrimidine) or 6-azauridine (XLVII) retard the bioactivation of 5-fluorouracil, thus decreasing not only its toxicity, but also in many cases its antitumor activity.

The most interesting development of the past few years in the study of antimetabolites was the discovery of thiazofurin, 2-β-D-ribofuranosyl-4-carbamoylthiazole (XLVIII), which exhibits very potent experimental antitumor activity [21]. In cells it forms an analog of NAD known as TAD, in which it takes the place of the nicotinamide nucleoside residue. TAD inhibits many important oxidation processes which are generally carried out by NAD, among them most importantly the biosynthesis of guanylic acid [4].

One of the five most important antitumor drugs in terms of frequency of use is methotrexate (methopterin, LIV) [1]. It acts as an inhibitor of dihydrofolate reductase, the enzyme involved in the biosynthesis of purines and pyrimidines. Although many analogs with valuable characteristics are known (for example, in the US a lipophilic analog capable of crossing the blood–brain barrier is widely used), still methotrexate is the only drug of this type which is administered clinically. A major discovery of the past decade related to the use of methotrexate therapy took place in the field of clinical oncology. It was found that if after treatment with large, superlethal doses of methotrexate, a patient was given the natural metabolite, 5-N-formyltetrahydrofolic acid (citrovorum factor, CF), it was possible to protect cells (particularly bone marrow cells) from the toxic effects of the methotrexate while maintaining the high level of effectiveness of the drug against the tumor cells [22]. The use of this type of rescue therapy (methotrexate and CF, or methotrexate and thymidine/inosine) is effective in the treatment of several types of tumor diseases which previously could not be controlled. In combination with metabolites, methotrexate may be introduced in very large doses, which greatly increases the value of the therapy. However, since it is necessary to use not only large quantities of methotrexate but also large amounts of the "rescue" drugs, CF, thymidine, and others, these must be administered in forms that can be tolerated by the patient in high doses. The use of high doses of methotrexate and "rescue" metabolites in cancer chemotherapy requires the evaluation and resolution of several technological and biological problems. An important finding in this regard concerns the treatment of tumors which are resistant to methotrexate. In several cases this drug induces the amplification of the target enzyme, thus increasing the production of the corresponding enzymes mentioned above [23].

The use of antimetabolites which affect nuclear function has contributed to an understanding of the processes involved in the transport, bioactivation, and catabolic degradation of these drugs. The study of these processes, in turn, has led to the preparation of more effective and biologically more stable compounds (second generation drugs). An example of this type of compound is 9-β-D-arabinosyl-2-fluoroadenine (XLIX), which, in contrast to ara-A, is not deaminated by adenosine deaminase, and which is, therefore, much more active [4]. The directed synthesis of antimetabolites will be possible when the molecular mechanisms involved in the malignant transformation of cells are completely understood.

It is traditional in a review of this type not to discuss both synthetic and natural antitumor agents. However, a distinction of this type is no longer scientifically warranted. In the first place, the division between synthetic and naturally occurring antitumor compounds is largely a semantic one; more and more natural products and their analogs are being synthesized, and semisynthetic compounds derived from other natural products are also being developed. Secondly, an important trend in contemporary cancer chemotherapy involves the use of antimetabolites and other bioregulators of natural origin. Thus, in the USSR as well as in the USA, antibiotics and other drugs derived from plants constitute one-quarter of the antitumor agents being used clinically [3]. Furthermore, in the USA, of all of the drugs undergoing various stages of preclinical evaluation in 1983, one-half were natural products or compounds obtained via their transformation [6].



LV, LVI, LVIII R=CH_3; LV $R^1=R^2=H$; LVI $R^1=OH$; $R^2=H$; LVII $R=R^1=R^2=H$; LVIII $R^1=OH$, R^2= tetrahydro-2-pyranyl; LX R=H; LXI R=OH

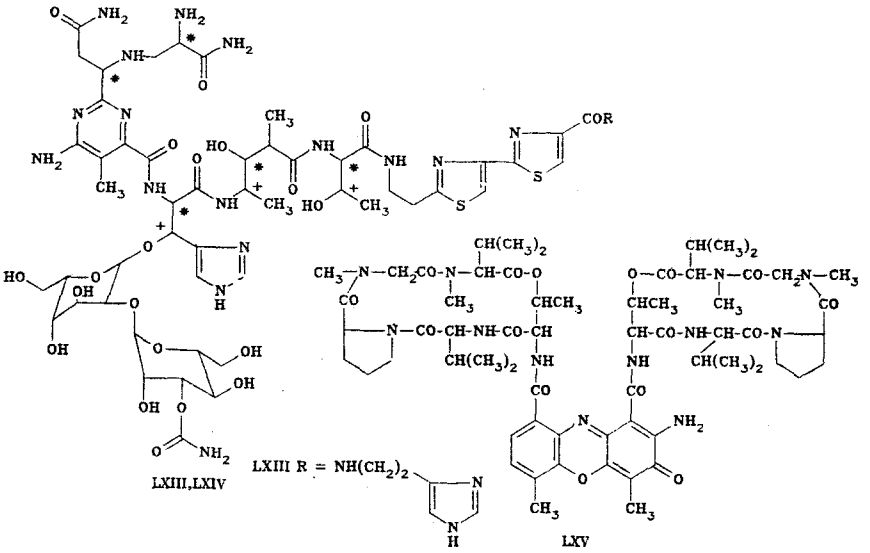
At the present time the most widely used drug in cancer chemotherapy is adriamycin (doxorubicin, LVI), which belongs to the anthracycline class of antibiotics. The first member of this class of antibiotics to be used medically was daunomycin (rubomycin, LV), which was obtained in 1963 [1]. Adriamycin, one of the most effective drugs, active against a large number of tumors, was first obtained by fermentation; later, a synthesis from daunomycin was developed. The original Soviet antibiotic karminomycin* (LVII) also belongs to this class of drugs, although it does not exhibit the wide range of applicability of adriamycin. These antibiotics are derivatives of anthraquinone aglycones (adriamycinone, daunomycinone, and karminomycinone, respectively), which do not possess antitumor activity, and the sugar (carbohydrate) daunosamine. Many studies are currently in progress to deduce the mechanism of action of drugs of this type. It is known that they are capable of reacting with DNA; a membrane effect also seems to be important in the case of adriamycin. One of the properties of adriamycin is its profound cardiotoxicity when used as part of an intensive therapy regime. A large amount of research is dedicated to the search for more active, less cardiotoxic adriamycin analogs, particularly in the USA, Italy, and Japan [24]. Several adriamycin analogs, especially amino- and carbonyl-group derivatives, are currently undergoing clinical trials. Worth noting in this regard is the 3'-O-tetrahydropyranyl derivative of adriamycin (THP, trerubicin,* XLVIII), which is absorbed very quickly by tumor cells [25].

A second type of anthracycline antibiotic, namely aclacinomycins, is not yet represented in medical practice, although aclacinomycin A (aclarubicin, LIX) is undergoing preclinical evaluation in the USSR [26]. The high reactivity of the anthracycline antibiotics has stimulated a large body of research in the anthraquinone series. The compounds mitoxantrone (LX) and ametantrone (LXI) are currently undergoing clinical trials in the USA [6]. The studies devoted to the preparation of semisynthetic antibiotics are expanding in the USSR as well.

The antibiotic bleomycin (LXIII) is another important drug used in the treatment of tumors. The structure of antibiotics in the bleomycin series was first delineated by Umezavoy and co-workers [25]. A total synthesis of bleomycin was then achieved. Bleomycin readily forms an equimolar complex with Cu^{2+}; this complex penetrates the cell nucleus, where the bleomycin is released to react with DNA. Bleomycin also forms a complex with Fe^{2+} and O_2 which is very important, since it induces cleavage of DNA molecules. More than 400 semisynthetic Bleomycin derivatives have been prepared in Japan. In the USSR the Soviet drug blemistetin* is approved for medical use; this material, which differs from bleomycin in its isomeric composition, is just as active, although slightly less toxic than the latter [27]. Peplomycin (LXIV), currently used in the USA, is an interesting semisynthetic derivative of bleomycin [25].

Actinomycin D (dactinomycin, LXV) is another antibiotic with important antitumor activity. Several naturally occurring actinomycins are known; they differ from one another in the structure of the peptide portion of the molecule. Only actinomycin D is applied in medical practice. It forms a complex with DNA which completely disrupts the processes of replication and transcription. Several models have been proposed for the reaction of DNA with actinomycin D; these models serve as the basis for the preparation of less toxic and more active analogs, as well as for a study of the mechanism of action of the drug with DNA [1].

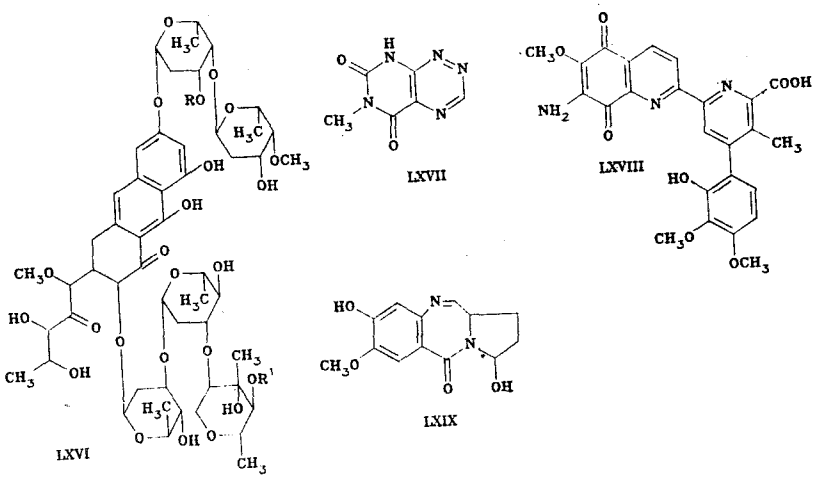
*See note p. 23.



LXIII R=NH(CH$_2$)$_3$SOCH$_3$(Al); R=NH(CH$_2$)$_3$SCH$_3$ (demethyl-2); R=NH(CH$_2$)$_3$S$^+$(CH$_3$)$_2$ (A2); R=NH(CH$_2$)$_4$NH$_2$ (A2′-a); R=NH(CH$_2$)$_3$NH$_2$ (A2′-B); R=NH(CH$_2$)$_3$NH(CH$_2$)$_4$NH$_2$ (A5); R=NH(CH$_2$)$_3$NH(CH$_2$)$_4$NH(CH$_2$)$_3$NH$_2$ (A6); R=NH$_2$ (B1′); R= =NH(CH$_2$)$_4$NHC(NH)NH$_2$ (B2); R=NH(CH$_2$)$_4$NHC(NH)NH(CH$_2$)$_4$NHC(NH)NH$_2$ (B4);
LXIV R=NH(CH$_2$)$_3$CH(CH$_3$)C$_6$H$_5$

Antibiotics belonging to the mitomycin series also exhibit cytotoxic properties; the aziridine, quinone, and urethane portions alkylate nucleic acids. Similarly, just as in the case of classical alkylating agents, their targets seem to be the guanylic fragments of the DNA chains. Mitomycin C (LXII) is used clinically [1, 3].

Discussions of the antitumor antibiotics would be incomplete without mention of representatives of the aureolic acid series, namely mitramycin (used in the USA), and olivomycin (used in the USSR). These compounds suppress the DNA-dependant synthesis of RNA. The drug olivomycin actually consists of a mixture of four isomers, olivomycin A, B, C, and D. Olivomycins A, B, and C differ in the acetyl and isobutyryl fragments of the hydrocarbon residue, whereas olivomycin D is missing the olivomycosyl unit [28]. Only one antibiotic of this class, variomycin, is currently undergoing clinical evaluation in the USSR [2].



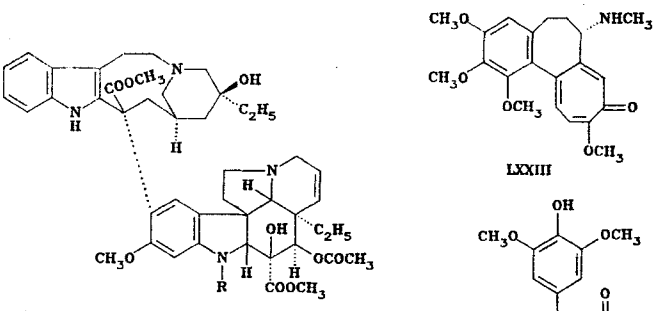
LXVI R=Ac, R^1=*i*-PrCO (A); R=Ac, R^1=Ac (B); R=H, R^1=*i*-PrCO (C)

An original Soviet antibiotic reumycin (LXVII) is involved in clinical trials in the USSR; this compound affects the oxidation of cytoplasmic NADH and disturbs the cell energetics [29]. The antitumor antibiotic bruneomycin (streptonigrin, LXVIII) is also approved for medical use in the USSR; the antitumor activity of this drug is also based on interaction with the DNA components of tumor cells [1, 3].

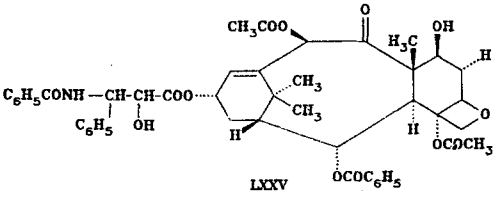
Antibiotics belonging to the pyrrolo[1,4]benzodiazepine group possess cytotoxic properties due to their reactivity with DNA; the least toxic compound of this series is neotramycin* (LXIX) [25]. Worldwide, many other types of antitumor antibiotics are being used; these include polypeptides (neocarcinostatin, largomycin [methacycline]), cyclic depsipeptides (didemin*), polyene derivatives (rapamycin), heterocyclic compounds (echinomycin*), nucleoside antibiotics, and others. This list of compounds illustrates the diversity of chemical approaches to the preparation of antitumor drugs and simultaneously points out the importance of DNA interaction for the presence of antitumor activity.

Current advances in research devoted to the preparation of antibiotic derivatives based on the accepted production of the known drugs may lead to valuable new pharmaceutical substances.

An important direction in cancer chemotherapy involves the use of enzymes to destroy compounds which are needed for the growth of tumor cells. The enzyme asparaginase (krasintin*) (L-asparagine aminohydrolase), which converts asparagine to aspartic acid, is an example of this type of drug to have found clinical application. It is being used (in combination with other drugs) in the treatment of severe leucoses. Asparaginase is obtained via the fermentation of *E. coli* and several other microorganisms [3].

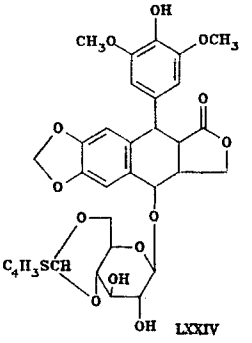


LXXI R=CH_3, LXXII R=CHO



LXXV

LXXIII

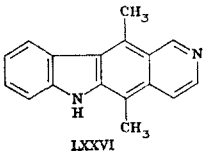


LXXIV

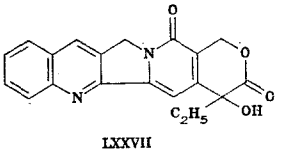
Research in the area of drugs derived from plants may be subdivided into two groups: the search for new cytotoxic substances (inhibitors of mitosis and DNA metabolism), and the search for immunomodulators. Several drugs are known to interact with the cytoskeleton and suppress mitosis. The most noteworthy compounds of this type are the Vinca alkaloids, which inhibit the polymerization of microtubular proteins of mitotic spindles. Vinblastine (LXXI) and vincristine (LXXII) are widely used in tumor therapy. These compounds are obtained from the *Vinca rosea* plant, which is cultivated in India. In the USSR this plant is also cultivated, and rosevin (Soviet vinblastine) is obtained; rosevin is approved for use in the USSR [3].

Other inhibitors of mitosyl colchamine (N-methyl-N-deacetylcolchicine, LXXIII) and teniposis (podophyll toxin derivatives, VM-26, LXXIV) are approved for use in the USSR [3]. The drug taxol (LXXV), which acts basically on the stabilization of the microtubules of mitotic spindles, is being studied in clinical trials in the USA [6].

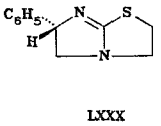
In a short review of this type it is difficult to discuss all of the different classes of alkaloids and plant derivatives which possess antitumor activity. Many studies have been devoted to the use of alkaloids related to ellipticine (LXXVI), camptothecin (LXXVII), and pyrrolizine derivatives (LXXVIII). Homoharringtonin (LXXIX) is used in both the USA and Korea. The mechanism of action of most antitumor antibiotics is linked to their interaction with DNA in tumor cells. Also interesting are studies of plant hemagglutinins, natural flavonoids, glycosides of natural steroids, and many others [2].



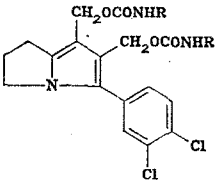
LXXVI



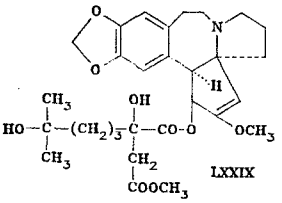
LXXVII



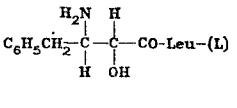
LXXX



LXXVIII R=CH(CH$_3$)$_2$,C$_6$H$_{11}$



LXXIX



LXXXI

One of the most rapidly developing fields of research in cancer chemotherapy is the search for substances which alter the reactions of organisms to tumors, for instance, immunomodulators [30, 31]. The first work in this area involved the clinical use of BCG vaccines, the bacteria *Corinebacterium parvum*, and the drug levomizol* (dekaris,* LXXX). Specific immunomodulators are used to stimulate the formation and activity of the components of the cellular immune response system (T-lymphocytes, NK-cells), and also to retard the formation of T suppressors. A great deal of research has focused on bestatine (LXXXI), polysaccharides, and retinoids. The limits of this review do not permit a full discussion of the many problems in cancer chemotherapy, among them the use of interferon and interferonogens, the questions in hormone therapy, and others. Each of these constitutes a large and highly specialized area of research. Notwithstanding the structural complexity and diversity of compounds currently constituting the arsenal of drugs used in cancer treatment, two clear and well-defined rules should be noted; all of the drugs affect either the nuclear metabolism of cells (either the drugs themselves, or their metabolic products, or, in the case of hormones and antihormones, complexes of the drugs with hormone receptors), or the processes of mitosis. Further research developments should lead to the preparation of more effective substances which interact with the already known cellular targets, and also, more importantly, to the discovery of new targets in cancer chemotherapy.

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*Limitations of time and resources have prevented a rigorous verification of the compounds mentioned in this article. Where the equivalent English for a common name could not be found, we have given a rough transliteration of the Russian name — Publisher.

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STRUCTURE OF 2-AMINO-4-THIAZOLINONE AND ITS 2-ARYL DERIVATIVES IN THE CRYSTALLINE STATE

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UDC 547.789.3:543.422.4

According to IR spectroscopic analysis, the compounds 2-amino-4-thiazolinone and 2-arylamino-4-thiazolinone exist in the amino form in the crystalline state.

The amino-imino tautomerism of 2-amino-4-thiazolinone (pseudothiohydantoin, Ia) and its 2-aryl derivatives IIa-e has been described in the literature [1-3]. Two independent groups of investigators concluded that in the case of the 2-arylamino-4-thiazolinones (IIa-e), the amount of the amino form A increased as the electron withdrawing nature of the benzene ring substituents was enhanced, and also upon solution of the compounds in hydroxylic solvents [2, 3]. Vibrational spectroscopic analysis of the compounds Ia and IIa-e in the crystalline state led to the opposite conclusion [1, 3-7]. In the present paper we attempt to clarify this contradiction.

X-ray structural analysis (XRA) of the compounds under investigation indicated that the C=N double bonds were extensively delocalized [8-15]. The presence of short hydrogen contacts (N–H•••N and N–H•••O) in the crystalline state enhanced the resonance effect, which is characteristic even of unassociated molecules [1, 2]. It has also been established that in the crystalline state certain cyclic amides exhibit a type B form of dimeric association [11, 12, 15].

Based on the hydrogen atom positions and the C–N bond lengths, the 5-phenyl analog of the pseudothiohydantoin Ib was found to exist in the amino form A with its accompanying resonance forms [10, 11]; these structures are sometimes referred to as "zwitterionic imino structures" [10]. The 5-phenyl analog of 2-phenylamino-4-thiazolinone IIf also exists in the amino form A [14]. With respect to pseudothiohydantoin itself (Ia), the authors [9] assigned it, appar-

Lensovet Leningrad Technological Institute, Leningrad. Translated from Khimiya Geterotsiklicheskikh Soedinenii, No. 1, pp. 32-37, January, 1985. Original article submitted June 15, 1984.

 0009-3122/85/2101-0024$09.50