Anticancer agents

76. ANTIMETABOLITES

Antimetabolites

The chemical structure of antimetabolites is similar to that of metabolites which participate in biochemical processes observed in the body.

This similarity enables the body to use them in biosynthesis instead of normal metabolites.

This replacement leads to the incorporation of antimetabolites into DNA or RNA and causes misreading.

The term antimetabolites is applied to two groups of drugs.

The first group comprises drugs which are built as nucleotide analogs into the biopolymers of cells (DNA, RNA).
This group consists mainly of pyrimidine and purine analogs and their nucleosides.

These analogs are incorporated into DNA and make its replication impossible or inhibit competitively the incorporation of deoxyribonucleoside trisphosphate into DNA.

The second group involves drugs inhibiting the synthesis of metabolites, necessary for cell life.

Their competetive action does not depend on their participation in the building of cellular biopolymers.

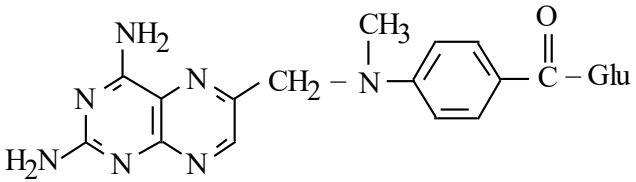
Inhibitors of mureine synthesis, nucleoside precursors, folic acid antagonists and tetrahydrofolic acid are classified into this group.

Three groups of antagonists are distinguished:

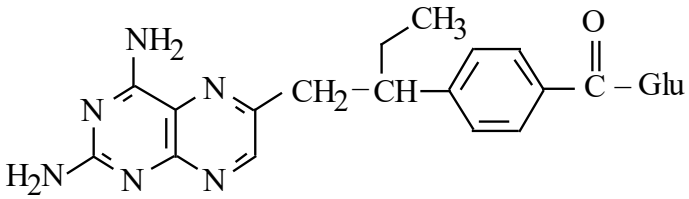
- Tetrahydrofolic acid
- Pyrimidine analogs
- Purine analogs

Antimetabolites of folic acid

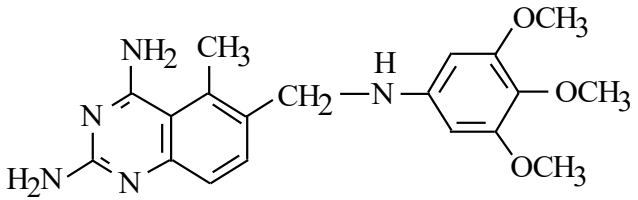
As antagonists of dihydrofolic acid which block the enzyme dihydrofolate reductase (DHFR) are classified some anticancer agents and immunosupressants:



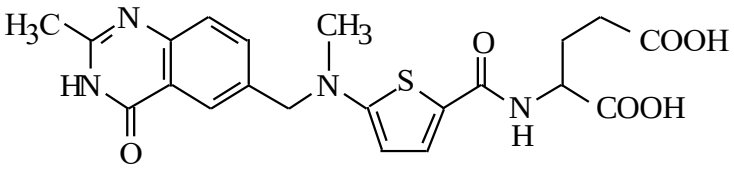
Methotrexate, METHOTREXAT, TREXAN



Edatrexate



Trimetrexate



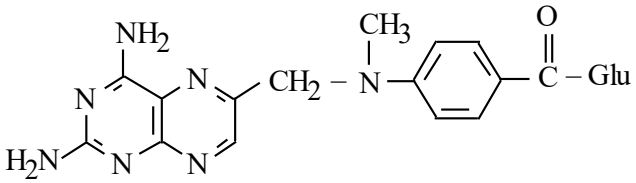
Raltitrexed, TOMUDEX

Methotrexate binds competitively with dihydrofolate reductase irreversibly. This bond is 10, 000 times stronger than with a normal metabolite.

The biological half-time of methotrexate is 3.5 h.

Methotrexate is used mainly in the treatment of leukemia, breast carcinoma, pulmonary carcinoma.

It permeates into the cerebrospinal fluid.

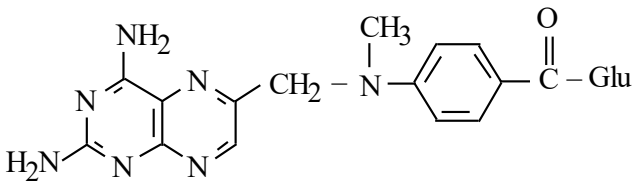


Methotrexate is a potent but very toxic drug.

Unwanted action affects the hematopoietic system, gastrointestinal tract and kidneys.

Nephrotoxicity is caused by a methotrexate metabolite – 2,4-diamino-*N*-methylpteroinic acid. It is created under the influence of bacterial peptidases present in the stomach.

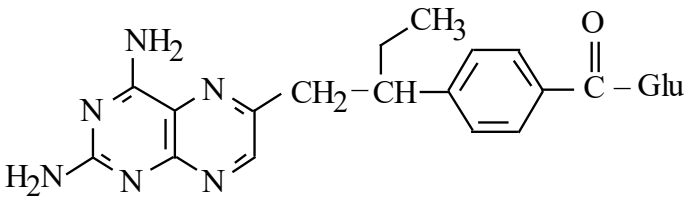
They cleave the glutamic acid rest from the parent compound.



Edatrexate demonstrates greater anticancer activity against cancers of various organs and lower toxicity than methotrexate.

Its greater activity is caused by the intensive intracellular metabolism of cancerous cells to polyglutamated forms.

Edatrexate is the most effective in the treatment of pulmonary microcellular carcinoma.

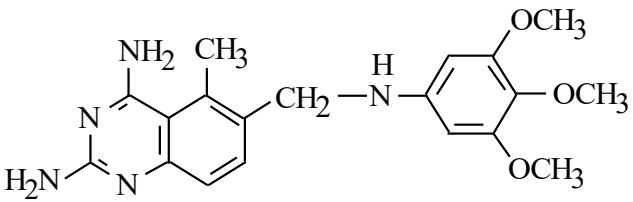


Trimetrexate - the analog of methotrexate - acts also in the S phase of the cell cycle.

It differs from the precursor only in the lack of a glutamic acid rest.

It acts synergistically together with fluorouracil and calcium folate.

It is effective in the treatment of colorectal carcinoma, breast carcinoma, head and stomach carcinomas and soft tissue sarcoma.

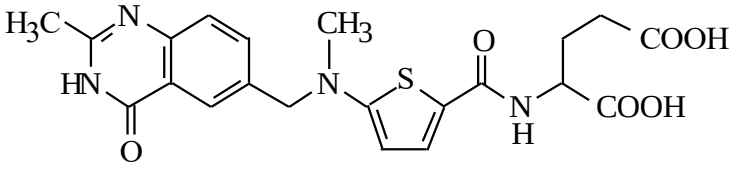


Raltitrexed is a new analogue of folates, inhibitors of thymidylate synthetase, which is necessary in the synthesis and repair process of DNA.

In the body it undergoes a rapid transformation to polyglutamated forms which act 100 times more strongly than the parent compound.

Polyglutamated forms act toxically on the normal cells.

Raltitrexed is effective in the treatment of colorectal and rectal carcinomas, ovarian carcinoma, breast carcinoma, pancreas carcinoma and non-microcellular pulmonary carcinoma.



Antimetabolites of pyrimidine analogues

Purine and pyrimidine analogs, the basic elements of the chemical structure of DNA and RNA, play the main role in their normal function.

Cytosine, thymine and uracil are pyrimidine analogs.

Those bases, bound with pentoses – D-ribose ((Ryb), 2-deoxyribose (d-Ryb) or cytosine (C) create nucleosides – cytidine, uridine, thymidine.

Those compounds create esters with phosphoric acid and are converted into ribonucleotides: uridine monophosphate (UMP), cytidine monophosphate (CMP), thymidine monophosphate (TMP).

If the sugar fragment of those nucleotides is 2-deoxyribose, those derivatives are called deoxythymidine monophosphate (dTMP) and deoxycytidine monophosphate (dCMP).

Synthetic fluorine derivatives of pyrimidine analogs and their nucleotides play an important role in cancer therapy.

Those antimetabolites act mainly as anticancer or antiviral agents and their activity is the strongest in the S phase.

They differ in the mechanism of action and toxicity.

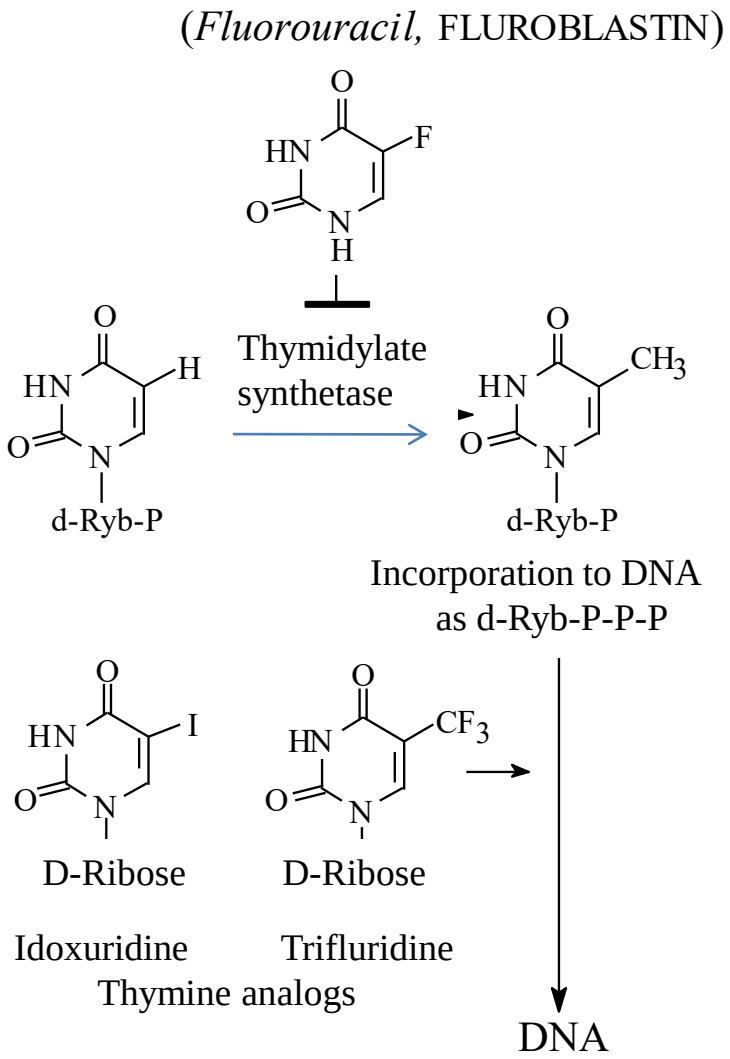
The mechanism of action depends on the size of substituents at position 5 in uracil.

5-Fluorouracil derivatives (5-FdUMP) inhibit the activity of thymidylate synthetase.

Their affinity for that enzyme is several thousand times greater than for natural dUMP.

5-FdUMP acts as an enzyme inhibitor and similarly to the natural substrate creates a covalently bound intermediate product.

This product is different from the natural substrate because of the presence of fluorine, which is why it is not cleaved into the enzyme and the end reaction product.



The action mechanism of pyrimidine analogs.

Derivatives with large substituents at position 5 (e.g.5-iodo- and 5-trifluoromethyl analogs of thymidine) are incorporated into DNA during biosynthesis.

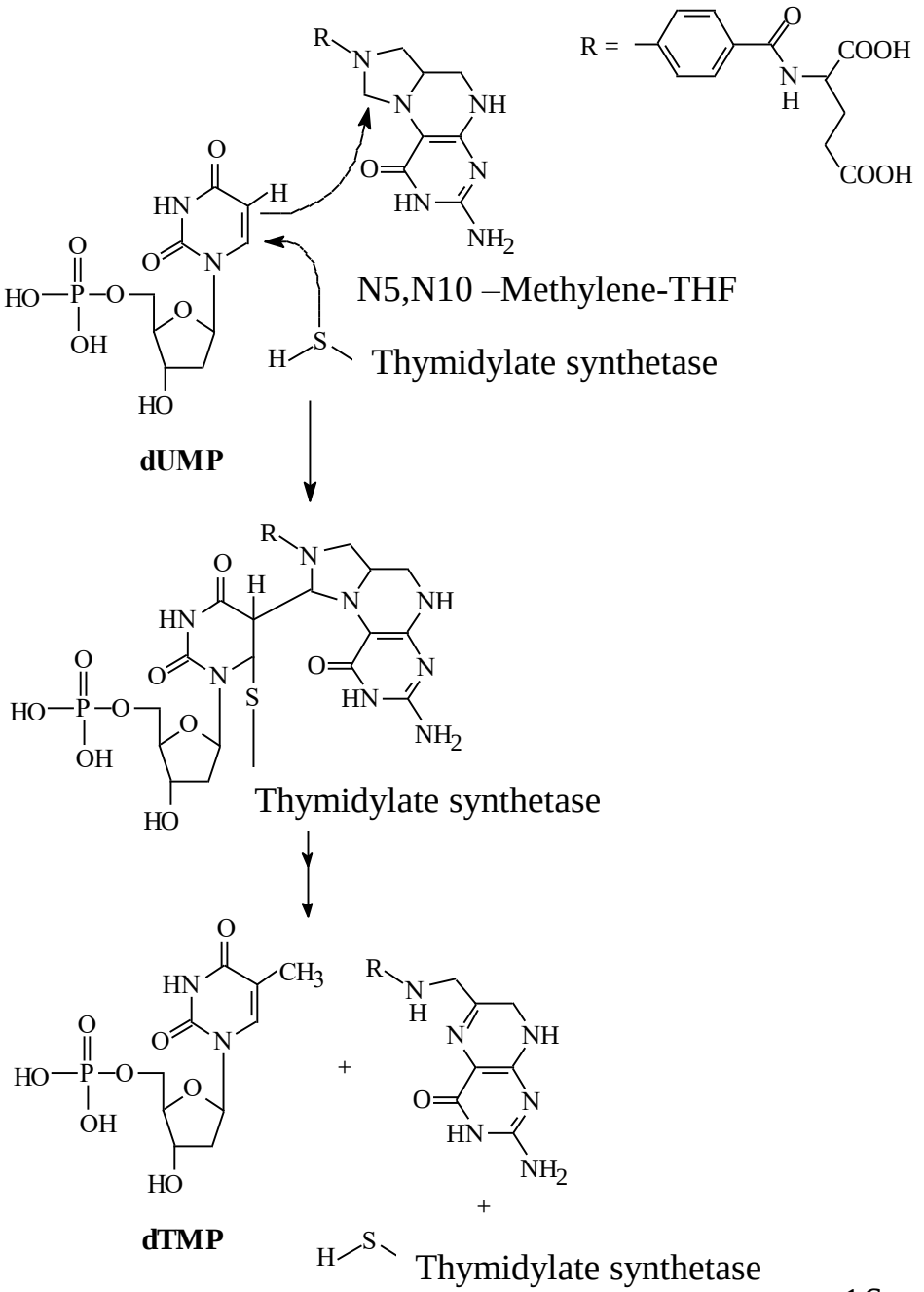
As a result of this process DNA functions are disturbed.

The inhibition of the ability of DNA to replicate decreases or interrupts the development of cells.

Idoxuridine and trifluridine, which is better soluble in water, are incorporated by viral and cellular DNA.

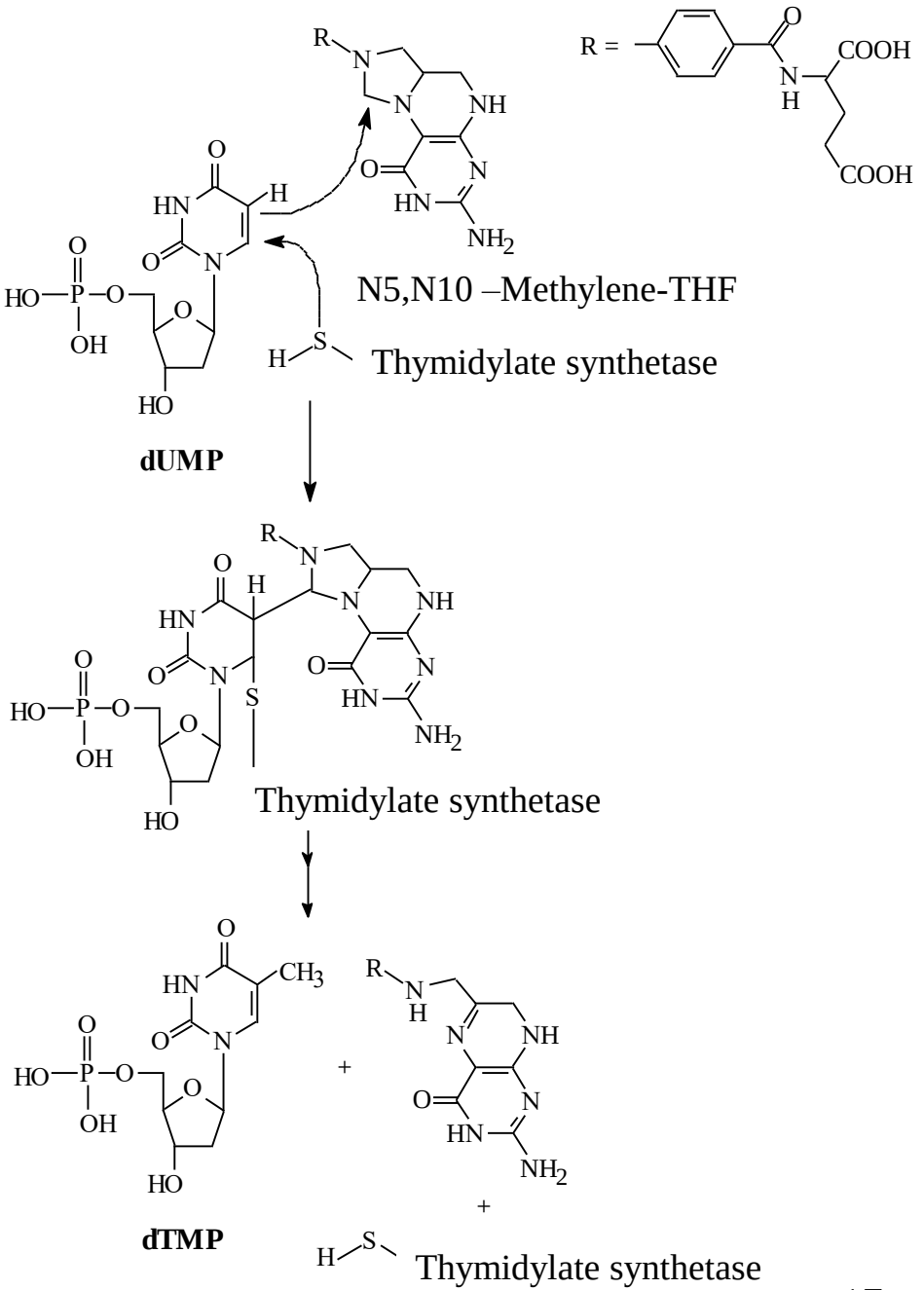
5-Fluorouracil (5-FU) was designed in 1957. The development of this compound was based on the observation that some tumors preferentially use uracil rather than orotic acid for pyrimidine biosynthesis.

Thymidine synthesis from uracil involves thymidylate synthetase and in this process a thiol group of a cysteine residue in the enzyme (E-SH) adds to the C-6 position of deoxyuridylic acid and with subsequent addition of the C-5 carbon to the N^5,N^{10}-methylene of N^5,N^{10}-methylenetetrahydro-folate.



The synthesis of deoxythymidylic acid from deoxyuridylic acid.

The resulting intermediate product transfers the C5-hydrogen to the N^{10} position of the folate creating deoxythymidylic acid, dihydrofolate, and the regenerated enzyme.



The synthesis of deoxythymidylic acid from deoxyuridylic acid.

In vivo, 5-FU must first be activated by conversion to 5- fluoro-2'-deoxyuridylic acid (5-FdUMP).

In general this occurs by conversion of 5-FU to 5-fluorouracil riboside which is then transformed directly into 5-FdUMP by ribonucleotide reductase.

The 5-FdUMP then binds to thymidylate synthetase to give an intermediate product that resembles the intermediate product formed with uridylic acid.

However, the intermediate compound has fluorine at C-5 instead of a hydrogen atom and the latter is unable to transfer the fluorine.

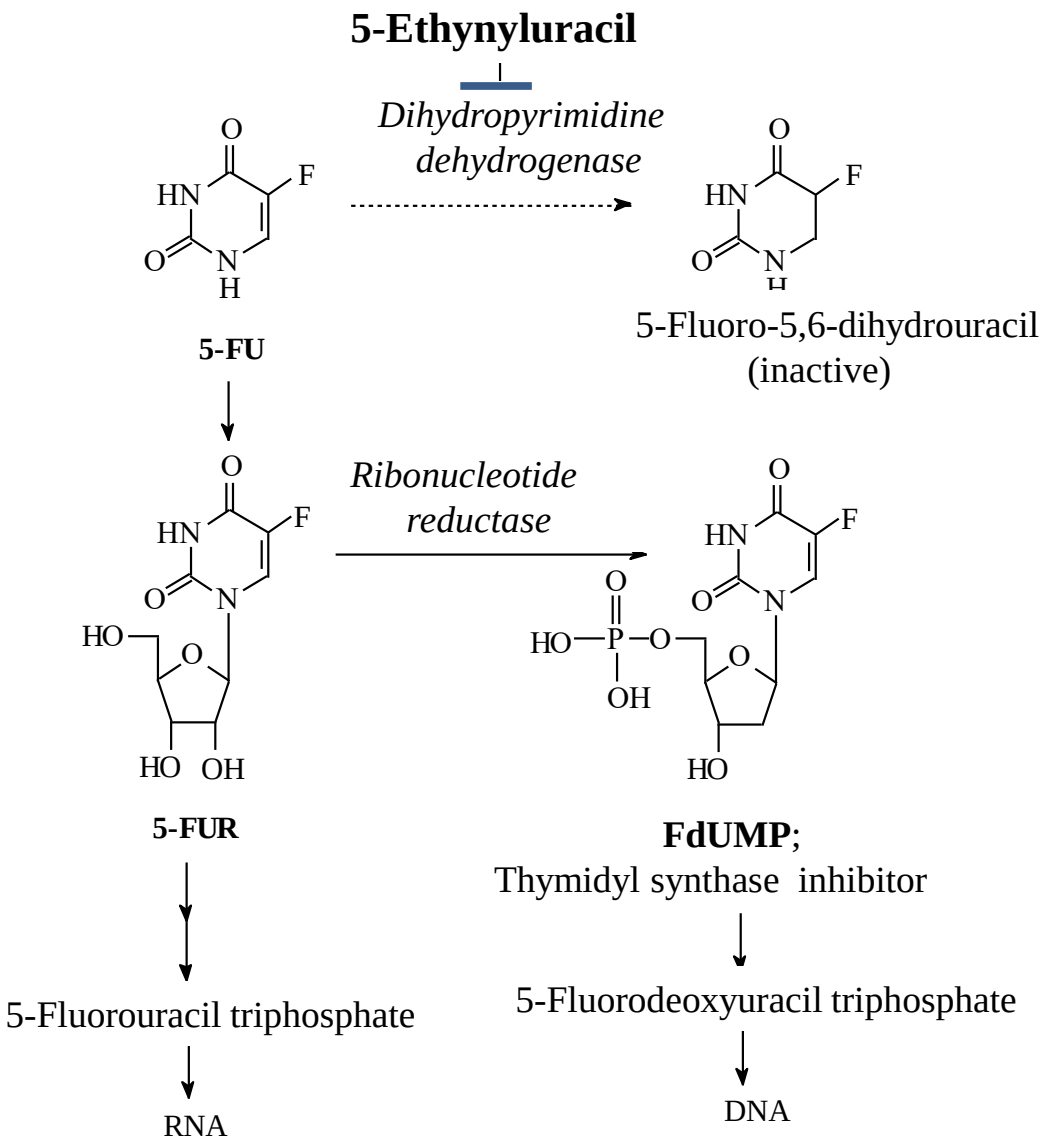
Thus, the intermediate compound cannot break down and the enzyme is inhibited.

This can lead to a deficiency of thymidine which is essential for the synthesis of DNA.

Additional metabolic reactions are thought to lead to the formation of 5-FU intermediate products which are incorporated into DNA and RNA and may contribute to the actions of the drug.

5-FU is also metabolized by dihydropyrimidine dehydrogenase leading to inactivated 5-fluoro-5,6-dihydrouracil.

This enzyme can be inhibited by 5-ethynyluracil which improves the therapeutic index of 5-FU by 2- to 4-fold.



The metabolism of 5-fluorouracil.

5-Fluorouracil is administered as intravenous infusion.

It can not be used orally because of its small biological availability caused by first-passage metabolism (enzymatic degradation under the influence of DPD – dihydropyrimidine dehydrogenase).

Long-term administration of intravenous infusion is troublesome for patients, may cause thrombus complications and creates a risk of infection.

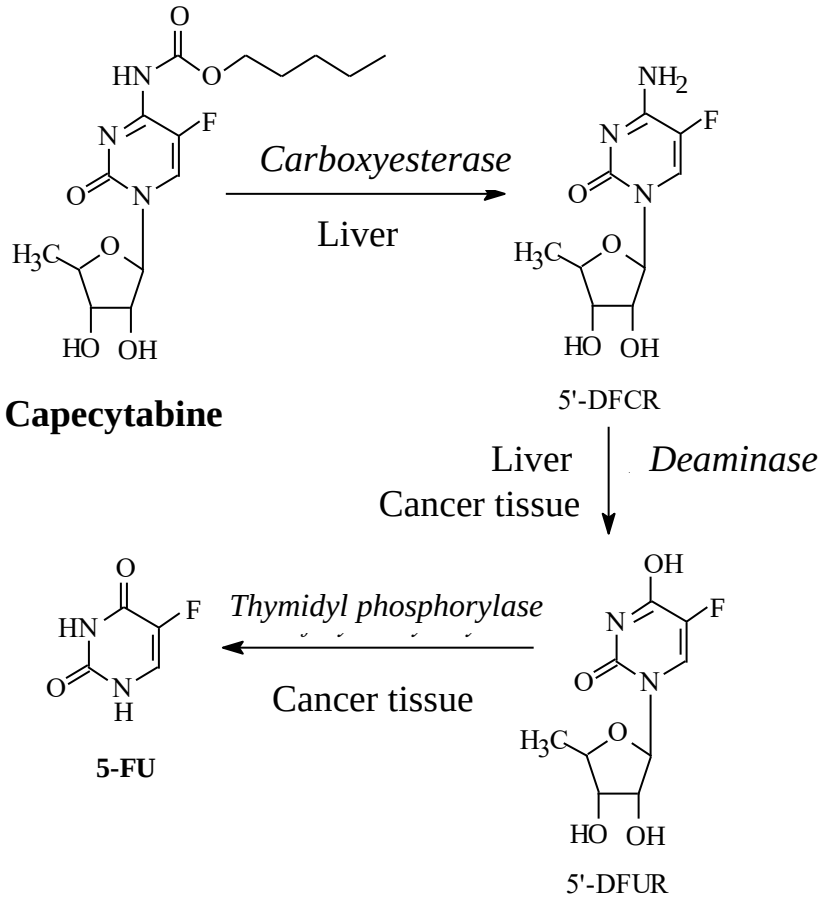
Two ways of administering active drugs into cancer cells are applied:

- **Administration of prodrug (capecytabine**), which is absorbed from the gastrointestinal tract in an unchanged form and next undergoes three-stage biotransformation to fluorouracil

- **Oral administration of fluoropyrimidine derivatives with a substance that inactivates the action of dihydropyrimidine dehydrogenase,** which eliminates first-passage metabolism in the wall of the gastrointestinal tract.

Because thymidyl phosphorylase occurs in greater concentrations in cancer tissue capecytabine acts the most selectively on cancer tissue compared to other fluoroderivatives of pyrimidine.

Capecytabine is used in the treatment of colorectal carcinoma and breast carcinoma.



5’-DFCR = 5’-deoxy-5-fluorocytidine
5’-DFUR = 5’-deoxy-5-fluorouridine

The biotransformation of capecytabine to 5-fluorouracil.

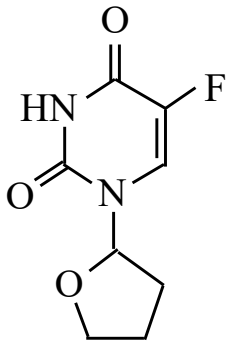
Tegafur is another prodrug which is transformed into 5-FU in the body.

Tegafur is more lipophilic and better absorbed from the gastrointestinal tract.

The preparations UFT and ORZEL contain tegafur and uracil.

Uracil is an inhibitor of dihydropyrimidine dehydrogenase (DPD).

They are used in the treatment of colorectal carcinoma, breast carcinoma and some head and neck carcinomas.



Tegafur, FTORAFUR

The preparation SI contains

- tegafur (prodrug),
- CDHP = chloro-2,4-dihydroxy-pyrimidine (inhibitor of DPD) and
- potassium oxonate (inhibitor of fluorouracil phosphorylation).

SI is an important drug in the treatment of colorectal carcinoma and stomach carcinoma.

Fluorouracil + Eniluracil (5-ethynyluracil)

Although eniluracil - a DPD inhibitor - does not demonstrate anticancer activity, it increases the biological availability of 5-FU and prolongs its biological half-life.

It is indicated in the treatment of breast carcinoma resistant to other drugs.

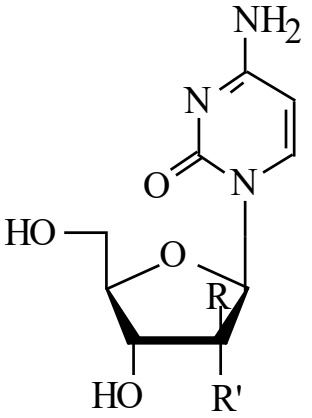
Thymine analogs (*floxuridine, idoxuridine, trifluridine*) are characterised by antiviral and/or anticancer reaction.

Their use is limited by their toxicity.

They are used in the treatment of

- stomach carcinoma,
- nasopharyngeal cavity carcinoma,
- urinary bladder carcinoma,
- ovarian carcinoma

Cytarabine and gemcitabine are pyrimidine derivatives with a nucleoside structure.



Cytarabine, R = -OH, R' = H; CYTOSAR

Gemcitabine, R = R' = F; GEMZAR

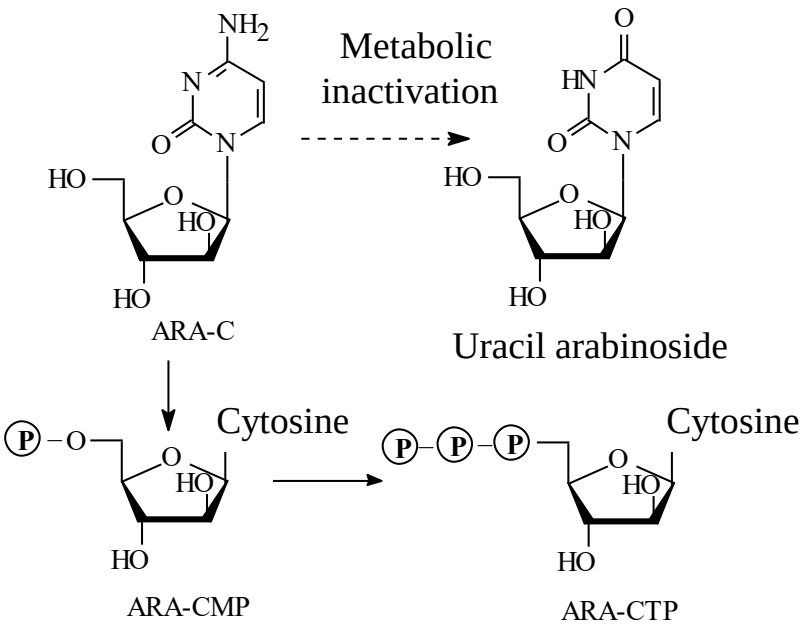
Cytarabine (ARA-C) is a cytosine analog in which the sugar has been modified.
In this case the sugar moiety is an arabinose (the OH group at position 2' has a β configuration) instead of ribose or deoxyribose.

Like 5-FU, ARA-C must be first converted into its monophosphate and then its triphosphate derivative (ARA-CTP).

This subtle change in the conformation of the 2'-carbon results in a compound that has multiple activities ARA-CTP:

- inhibits the conversion of cytidylic acid to 2'-deoxycytidylic acid
- inhibits DNA-dependent DNA polymerase.

Finally, ARA-C causes miscoding after being incorporated into DNA or RNA.



The metabolic activation and inactivation of ARA-C

Cytarabine is used in the treatment of acute lymphoblastic leukemia and myeloid leukemia, lymphomas and as immunosupressant.

Cytarabine permeates into the cerebrospinal fluid.

Its biological half-time is 2-3 h.

Cytarabine is administered as an intravenous injection.

After oral administration the desired concentration in the organs is not achieved because of the first-passage effect in the liver.

It is also possible to use it as a subcutaneous injection by means of an infusion pump.

Pyrimidine and purine nucleosides demonstrate the same mechanism of action, which is similar to that of gemcitabine.

Gemcitabine and other nucleosides are transformed into mono-, di- and triphosphate analogs under the influence of **deoxycytidyl kinase**.

The diphosphate derivative blocks ribonucleosidoreductase causing a decrease in the concentration of deoxycytidylotriphosphate (dCTP).

It has a beneficial influence on the incorporation of triphosphate as a false base into DNA resulting in the interruption of DNA biosynthesis and the death of cells.

A decreased cell supply of dCTP that is caused by inhibition of ribonucleosidoreductase releases a number of spontaneous mechanisms which are characteristic of gemcitabine.

As the concentration of dCTP decreases the amount of gemcitabine incorporated into DNA increases.

The inhibition of dCTP activity by deoxycytidylkinase increases the phosphorylation of gemcitabine to di- and triphosphate analogs.

These compounds, under the influence of deaminase, undergo deamination to inactive derivatives of uridine. dCMP-deaminase responsible for deamination is inhibited directly by gemcitabinotriphosphate and indirectly as a result of the decreased level of intracellular dCMP.

Gemcitabine inhibits the rebuilding and cellular deamination of its own active metabolites as a result of spontaneous action.

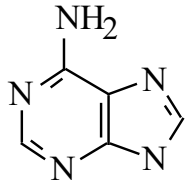
Gemcitabine slightly binds with protein and its half-time is 8 min.

It is deaminated under the influence of deaminase to 2’-deoxy-2’2’-difluorouridine (dFdU), whose half-time is 14 h.

The main indication for the use of gemcitabine is advanced pancreas neoplasm and the multicellular form of bronchial neoplasm, breast carcinoma and ovarian carcinoma.

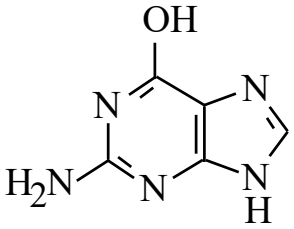
Purine antimetabolites

Purine bases like pyrimidine bases are responsible for the normal function of DNA and RNA in the transfer of genetic information.



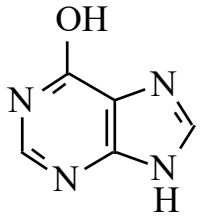
Adenine

Adenine and guanine are indispensable in DNA and RNA synthesis.



Guanine

They can bind with pentoses eg. d-ribose (Rib) or with 2-deoxyribose (d-rib) and create nucleosides – adenosine and guanosine.



Hypoxanthine

These compounds may create ester connections with phosphoric acid and transform into ribonucleotides – adenosine monophosphate (AMP) and guanosine monophosphate (GMP).

The sugar part of these ribonucleotides may also be 2-deoxyribose and then such compounds are called deoxyadenosine monophosphate (dAMP) and deoxyguanosine monophosphate (dGMP).

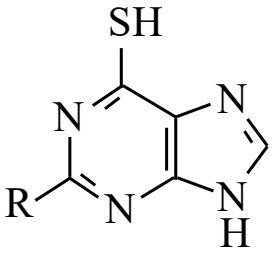
Purine antimetabolites may interfere with the biosynthesis of purine in cells.

They act during different stages of purine biosynthesis and decrease the concentration of purines in cells, which inhibits their development.

In medicine the most often used purine antimetabolites are 6-mercaptopurine and 6-thioguanine.

6-Thioguanine is a structural analog of guanine.

6-Mercaptopurine is the antimetabolite of hypoxanthine in which the –OH group at C6 was replaced by an –SH group.



Mercaptopurine, R = H

MERCAPTOPURINUM, PURINETHOL

Tioguanine, R = -NH_2; LANVIS

6-MP is converted *in vivo* to the corresponding ribonucleotide 6-thioinosinate by the enzyme hypoxanthine-guanine phosphoribosyltransferase (HGPRT).

6-Thioinosinate has been shown to be a powerful inhibitor of the conversion of 5-phosphoribosylpyrophosphate into 5-phosphoribosylamine, involved in purine biosynthesis.

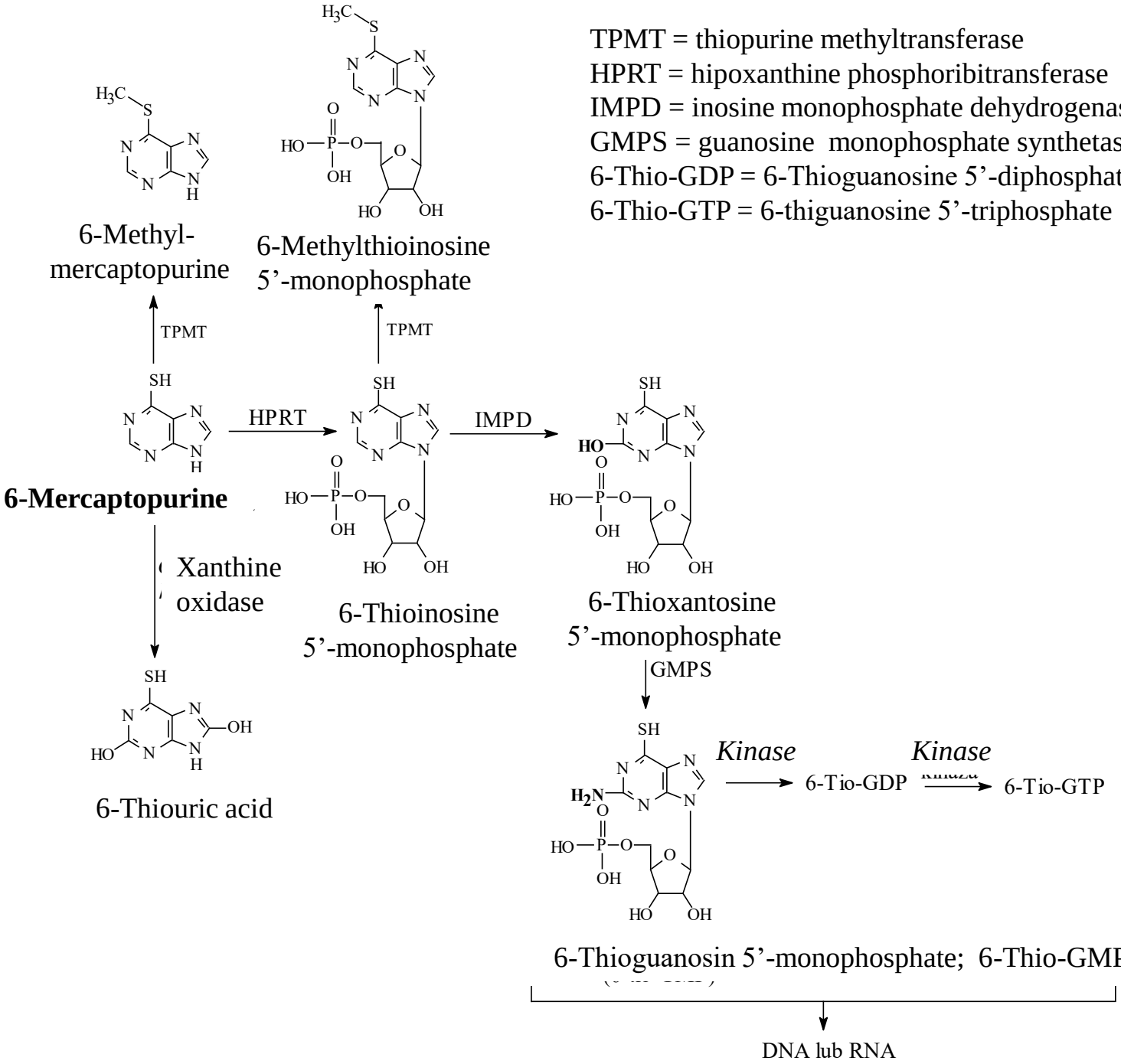
In addition, 6-thioinosinate inhibits the conversion of inosinic acid to adenylic acid as well as the oxidation of inosinic acid to xanthylic acid.

Therefore, the overall action of 6-MP is inhibition of the *de novo* synthesis of purines.

The mechanism of action of 6-MP and its metabolite 6-thioinosinate is only part of the activity as 6-thioinosinate can be further transformed into its ribose diphosphate and triphosphate.

Both of these species are enzyme inhibitors and the triphosphate can be used in DNA and RNA synthesis in place of guanine and after incorporation it inhibits chain elongation.

Finally, 6-thioinosinate can be a substrate for 3-adenosylmethionine and is converted to 6-methylthioinosinate which is responsible for some of the antimetabolite properties of 6-MP.


TPMT = thiopurine methyltransferase
HPRT = hipoxanthine phosphoribitransferase
IMPD = inosine monophosphate dehydrogenase
GMPS = guanosine monophosphate synthetase
6-Thio-GDP = 6-Thioguanosine 5'-diphosphate
6-Thio-GTP = 6-thiguanosine 5'-triphosphate
6-Methyl-mercaptopurine
6-Methylthioinosine 5'-monophosphate
TPMT
TPMT
6-Mercaptopurine
HPRT
IMPD
Xanthine oxidase
6-Thioinosine 5'-monophosphate
6-Thioxantosine 5'-monophosphate
GMPS
6-Thiouric acid
Kinase
Kinase
6-Tio-GDP
6-Tio-GTP
6-Thioguanosin 5'-monophosphate; 6-Thio-GMP
DNA lub RNA

6-MP undergoes intestinal, hepatic and intracellular metabolism.

The intracellular biotransformation of 6-MP is a result of two catabolic and one anabolic reactions.

The catabolic reactions - S-methylation (the main catabolic reaction of 6-MP) and oxidation of 6-MP to inactive 6-tiouric acid under the influence of xanthine oxidase - compete with the anabolic reaction – which involves the creation of active nucleotides of 6-thioguanine (Fig. 76.7).

6-Methylothioinosinate 5'-monophosphate is a very strong inhibitor of the *de novo* synthesis of purines.

Because its role *in vivo* has not been explained yet it is possible that it mediates in many biological processes.

Drug concentration is the main factor which influences the creation of 6-MP metabolites.

At low concentrations, the first metabolite was 6-MP nucleotide – 6-thioinosinate-5’-monophosphate (TIMP).

At greater concentrations of 6-MP (> 50 mM), oxidation metabolites and methyl derivatives of thionucleotides are observed.

The greater role of catabolic reactions resulting from increased drug concentrations *in vitro* may be important for therapy.

6-Thioguanine, like 6-MP, is first ribosylated to monophosphate (6-TGMP) and is then converted into the diphosphate derivative (6-TGDP) and triphosphate (6-TGTP).

Each of these intermediate forms of 6-TG inhibits a range of enzymes and processes that generally parallel the activity previously observed for 6-MP.

In addition, the ribosylated triphosphate of 6-TG can be incorporated into RNA, or after reduction to the 2'-deoxy derivative, into DNA.

After incorporation into DNA, 6-TG may inhibit DNA replication because of the inability of the replication enzymes to recognize 6-TG.

Generally, 6-TG parallels the activity of 6-MP, although thioguanine deactivation is not dependent upon xanthine oxidase.

These purine antimetabolites are mainly used in the treatment of leukemia in children and of choriocarcinoma. 6-MP is preferred in spite of its toxicity.

Mercaptopurineribonucleotide is an active form of 6-MP.

The half-time of 6-MP is 45-90 min.

It undergoes biotransformation in the body. Some of its metabolites are active (6-methylmercaptopurine) and some are responsible for unwanted effects.

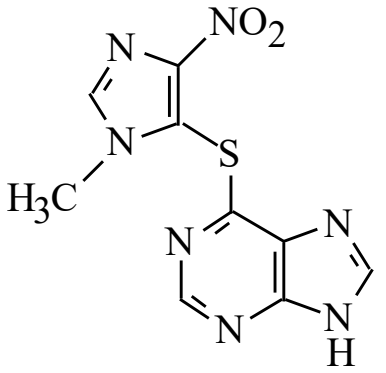
For example, when 6-MP is used for a long time, uric acid derivatives may accumulate in the body, which may lead to gouty diathesis.

In this case allopurinol is also used, which blocks xanthine oxidase. In the presence of allopurinol the half-time of 6-MP elimination is elongated, which allows decreasing the dosage of 6-MP by about 25%.

Thioguanine, like 6-MP, is used in the treatment of leukemia.

Azathioprine is a derivative of 6-MP.

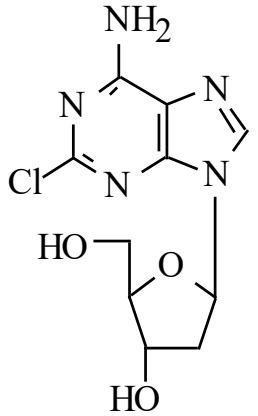
Azathioprine is a pro-drug which is easily converted in the body into active mercaptopurine, which is facilitated by the presence of an electrophilic substituent (imidazole derivative).



Azathioprine, IMURAN

Azathioprine demonstrates strong immunosuppressive action.

Cladribine and fludarabine phosphate are also purine antimetabolites structurally related to nucleosides.



Cladribine, LEUSTATIN

They are purine analogs resistant to adenosyl deaminase.

Cladribine undergores intracellular transformation to active phosphate metabolites, which accumulate mainly in lymphocytes – cells with great deoxycytidyl kinase activity.

In the cells that are proliferating cladribine inhibits the activity of **ribonucleotide reductase and DNA polymerases** and is incorporated into the DNA chain, interfering with its structure.

Cladribine damages cells in the rest phase. It induces the mechanism of apoptose, which leads to the severing of both DNA strands.

As a result, reconstructive processes are started and the existing amount of NAD is reduced.

ATP synthesis is inhibited, which disturbs the energetic processes of cells and causes their death.

Cladribine is administered IV because only 50% of it is absorbed from the gastrointestinal tract.

The biological half-time of cladribine is approx. 7 h.

Like other purine metabolites it is effective in lymphatic leukemia and in hairy cell leukemia. It is also active in acute and chronic myeloid leukemia and malignant astrocytoma.

The main adverse effect of cladribine is the damage of the hemopoietic system (granulocytopenia).

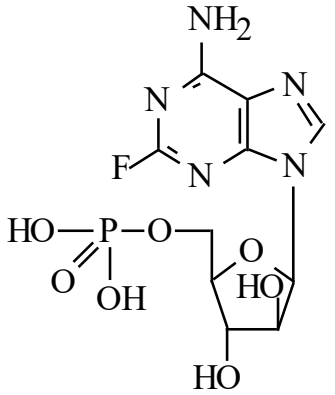
Kidney damage, gastrointestinal and CNS disturbances (headache, dizziness, paresthesia, paralysis of the lower limbs, insomnia) are also observed.

Fludarabine phosphate inhibits the activity of DNA polymerase and ribonucleotide reductase, resulting in the inhibition of DNA, RNA and protein synthesis.

This drug demonstrates the greatest activity in the S phase of the cell cycle. It is the most effective in the treatment of chronic lymphatic leukemia and non-Hodgkin lymphoma with a small degree of malignancy.

The main adverse effects include: damage of bone marrow, nephrotoxicity, hepatotoxicity, disturbance of the gastrointestinal function/activity.

Neurotoxicity may also appear (encephalopathy, consciousness disturbance, sleepiness, headache, spastic and flaccid paralysis, vision disorder), for which the metabolite of fludarabine (2-fluoro-ATP) is responsible.



Fludarabine phosphate
FLUDARA