Anti Metabolite Theory

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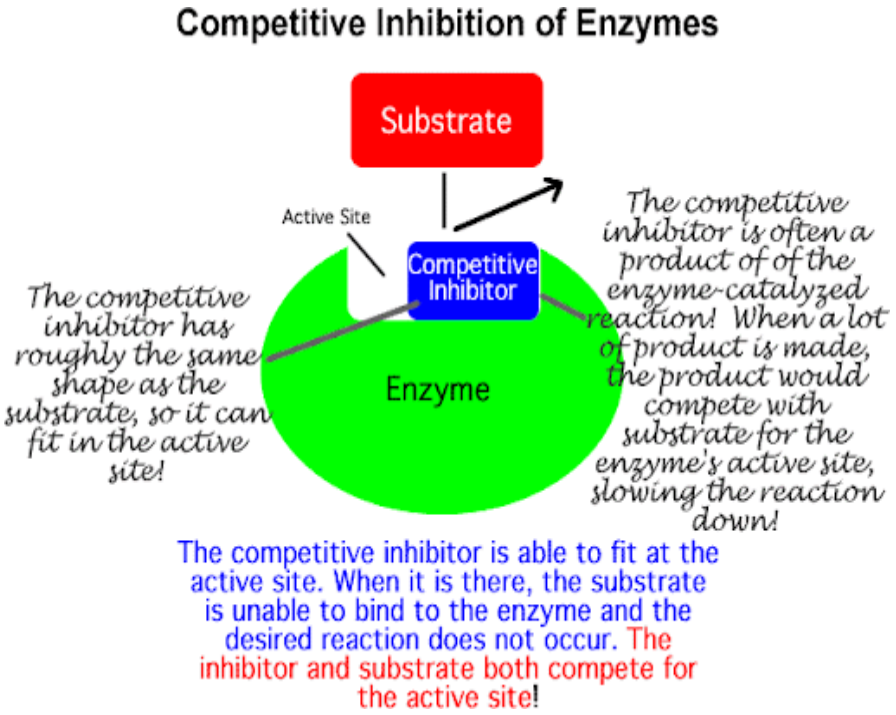
Anti Metabolite Theory

- Inhibition of the key element controlling the metabolic process would decrease or completely inhibit the synthesis of the intracellular key molecules.

- Changing the natural substrate of the enzymes involved in nucleotide and nucleic acid metabolism causes inhibition of metabolic processes. The development of this logic has increased the importance of the antimetabolite theory

In competitive inhibition the inhibitor is very similar in shape to the normal substrate. It binds to the active site to form an inhibitor-enzyme complex. This reduces the number of enzyme molecules available for the substrate molecules to bind to. As a result less catalysis takes place and so the rate of the reaction slows down



Allopurinol,

- Allopurinol, or 1,5-dihydro-4H-pyrazolo[3,4-d]pyrimidin-4-one, was one of the crown jewels of the venerable drug discovery programThe prototypical xanthine oxidase (XO) inhibitor allopurinol, has been the cornerstone of the clinical management of gout and conditions associated with hyperuricemia for several decades.

- More recent data indicate that XO also plays an important role in various forms of ischemic and other types of tissue and vascular injuries, inflammatory diseases, and chronic heart failure.

- Allopurinol and its active metabolite oxypurinol showed considerable promise in the treatment of these conditions both in experimental animals and in small-scale human clinical trials. Although some of the beneficial effects of these compounds may be unrelated to the inhibition of the XO, the encouraging findings rekindled significant interest in the development of additional, novel series of XO inhibitors for various therapeutic indications.
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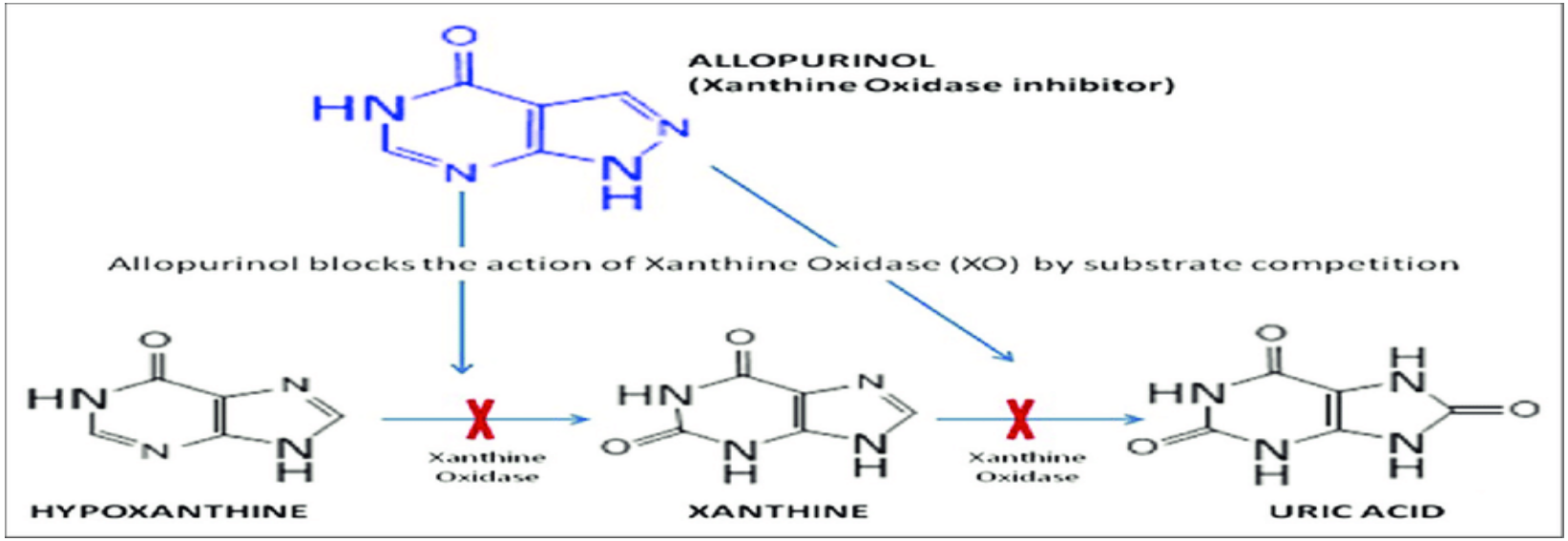


6-Mercaptopurine (6-MP)

Studies of Elion carried out in 1951 on 6-mercaptopurin (6-MP), which is a purine analog, are pioneering studies in use of antimetabolites in cancer treatment

Thiopurine drugs ; 6-mercaptopurine (6-MP), 6-thioguanine, and azathioprine—are used in the treatment of leukemia, autoimmune disorders, and solid tumors, as well as in organ transplantations. Among these purine and pyrimidine metabolites, 6-MP is the most prescribed drug for acute lymphoblastic leukemia (ALL).

- **Gertrude Elion (1918 – 1999) was an American biochemist and pharmacologist, who shared the 1988 Nobel Prize in Physiology or Medicine with George H. Hitchings and Sir James Black** for their use of innovative methods of rational drug design for the development of new drugs.

Her work led to the creation of the AIDS drug AZT. Her well known works also include the development of the first immunosuppressive drug, azathioprine, used to fight rejection in organ transplants, and the first successful antiviral drug, acyclovir (ACV), used in the treatment of herpes infectionBy 1951,

«we had made and tested over 100 purines in the L. casei screen. and <u>discovered that the substitution of oxygen by sulfur at the 6-position of guanine and hypoxanthine produced inhibitors of purine utilization.</u> 6-Mercaptopurine (6-MP) and 6-thioguanine (TG) were tested at the SloanKettering Institute, with whom we had estabished a collaboration, and were found to be active against a wide spectrum of rodent tumors and leukemias.»

THE PURINE PATH TO CHEMOTHERAPY

- **Nobel Lecture, December 8, 1988**
- **By GERTRUDE B. ELION**

6-Mercaptopurine (6-MP)

Mechanism of Action

i. Mercaptopurine competes with hypoxanthine and guanine which are purine derived structures, for the **HGPRT enzyme. Mercaptoporine is then converts into thio inosin monophosphate.**

ii. Thio inosine monophosphate **(TIMP) inhibits conversion of inosinic acid to xanthylic acid and adenylic acid through adenyl succinate.**

iii. Methylation of thio inosine monophosphate forms **6-methylthioinosinate (MTIMP).**

iv. Glutamine-5-phosphoribosylpyrophosphate amidotransferase is the enzyme requires for the purine ribonuceoltide synthesis. **This enzyme is inhibited by thio inosine monophosphate and MTIMP.**

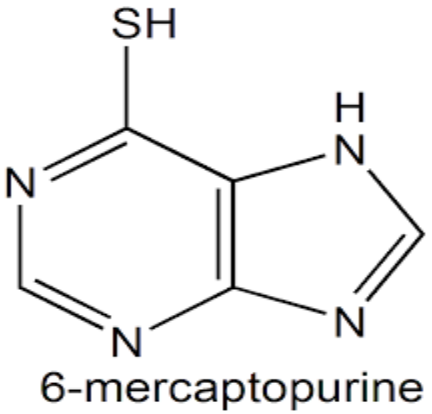
v. Since, **Glutamine-5-phosphoribosylpyrophosphate amidotransferase is rate limiting** factor for purine synthesis, this alres the synthesis and functioning of the RNA and DNA.

vi. Thus, 6-Mercaptopurine interferes with synthesis of glycoprotiens and interconversion of nucleotides. Therapeutic Uses

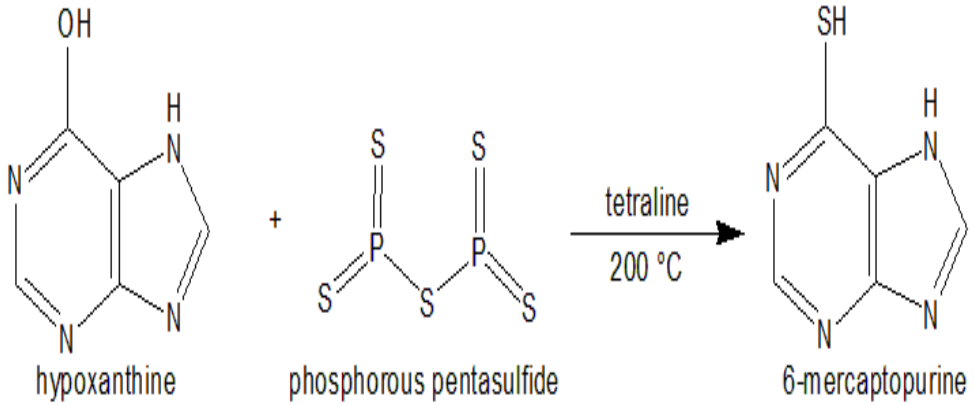
Acute lymphoblastic leukemia

Ulcerative colitis

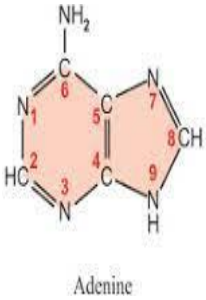
Crohn's disease



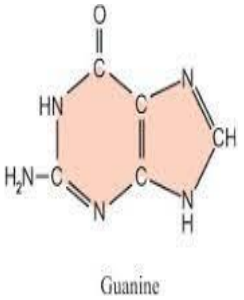
6-mercaptopurine



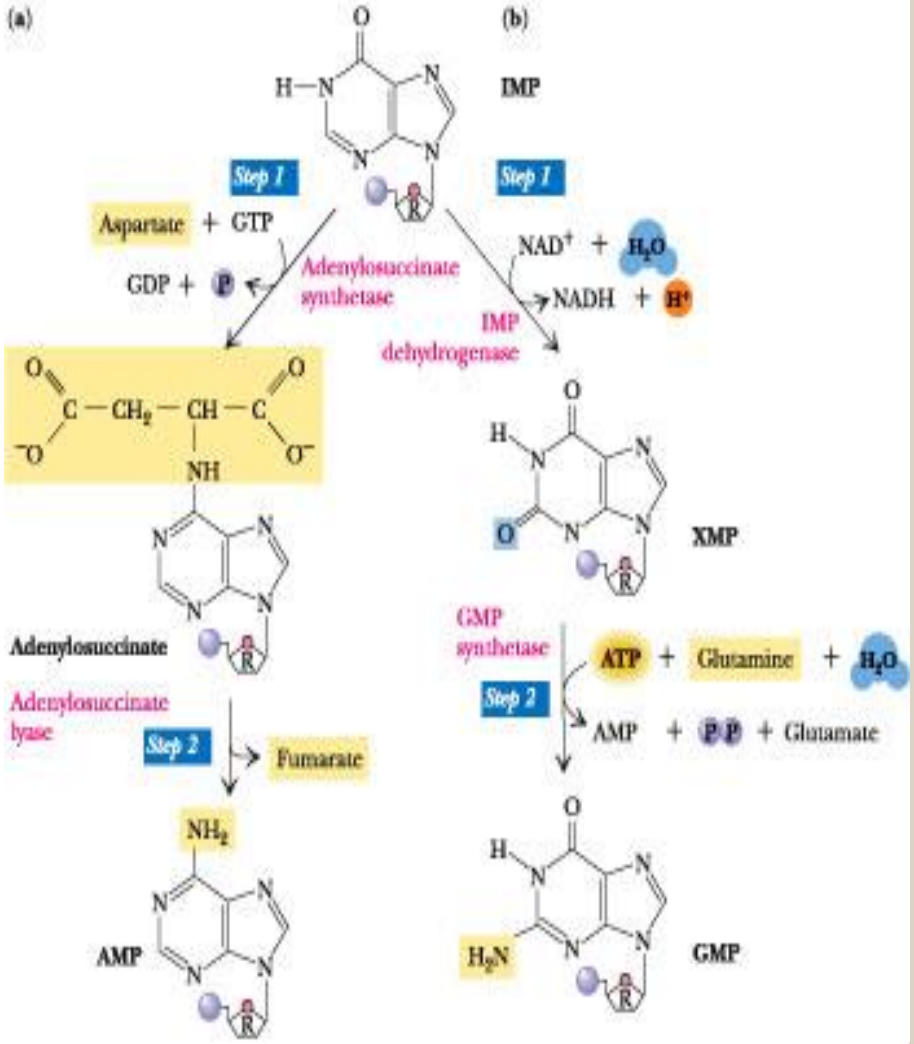
hypoxanthine phosphorous pentasulfide 6-mercaptopurine

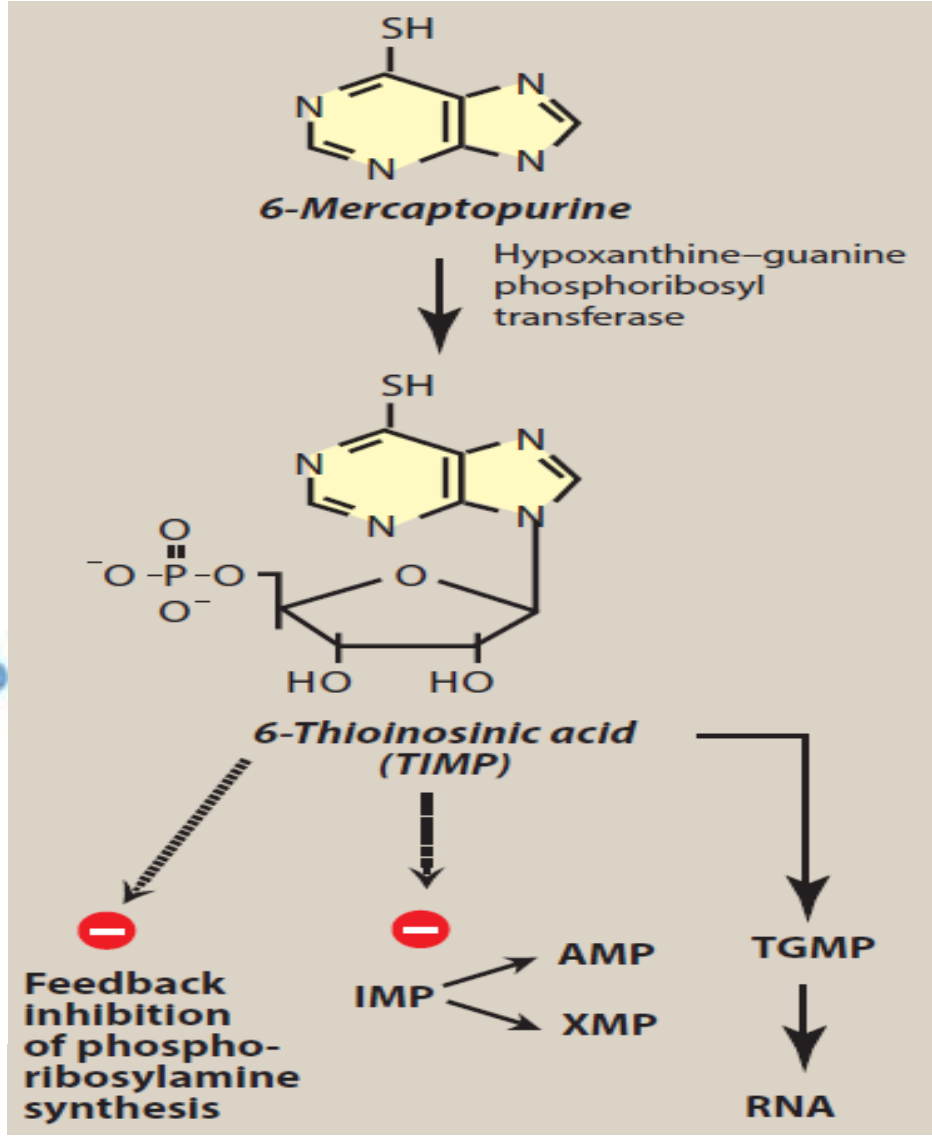


Adenine



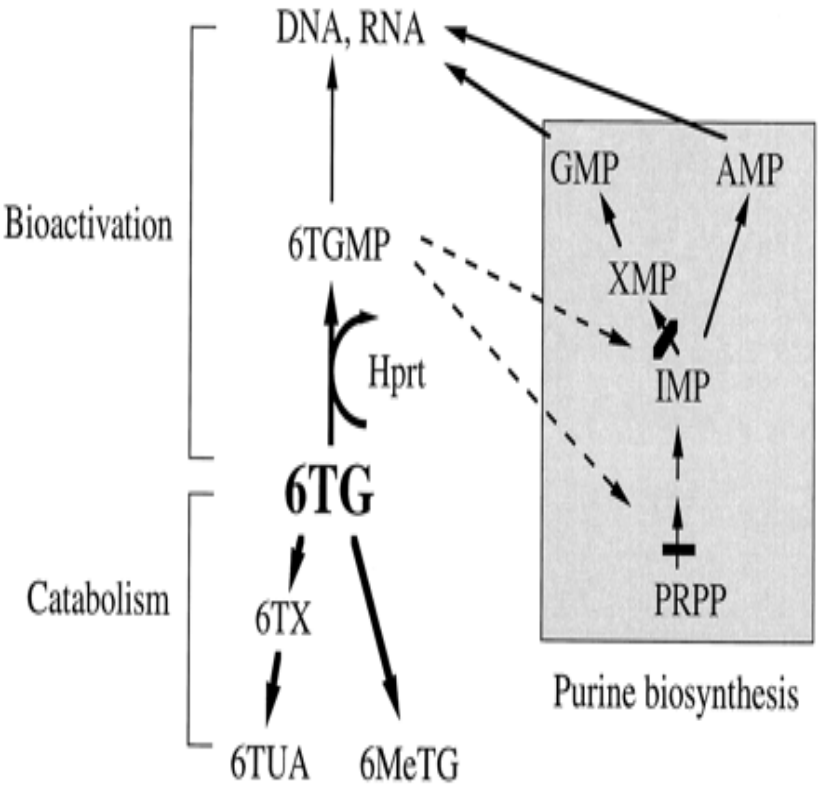
Guanine


(a)
(b)
IMP
Step 1
Aspartate + GTP
GDP + P
Adenylosuccinate synthetase
Step 1
NAD+ + H2O
NADH + H+
IMP dehydrogenase
XMP
Adenylosuccinate
Adenylosuccinate lyase
Step 2
Fumarate
GMP synthetase
ATP + Glutamine + H2O
Step 2
AMP + PP + Glutamate
AMP
GMP


6-Mercaptopurine
Hypoxanthine–guanine phosphoribosyl transferase
6-Thioinosinic acid (TIMP)
Feedback inhibition of phospho-ribosylamine synthesis
IMP
AMP
XMP
TGMP
RNA

6-THİOGUANİNE (6TG)

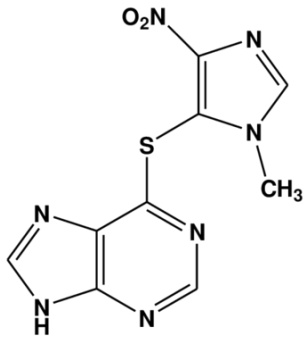
- **Mechanism of action and metabolic fate of 6-thioguanine.**
- **6TG is converted to its monophosphate (6TGMP) by Hprt**.
- This active metabolite interferes with de novo synthesis of purines by **pseudofeedback inhibition of phosphoribosyl pyrophosphate (PRPP).**
- Furthermore, 6TGMP is phosphorylated to triphosphate **(6TGTP) and incorporated into DNA and RNA.**
- The biodegradation of 6TG mostly of deamination and oxidation to inactive metabolites **6-thioxanthine (6TX) and 6-thiouric acid, respectively.** Small portion of 6TG also excreted in the form of 6-methyl-thioguanine (6MeTG).



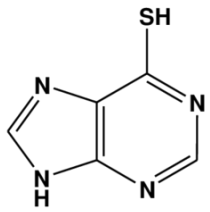
Azathioprine (AZA)

- It is an imidazolyl derivative of **6-mercaptopurine and** many of its biological effects are similar to those of the parent compound.

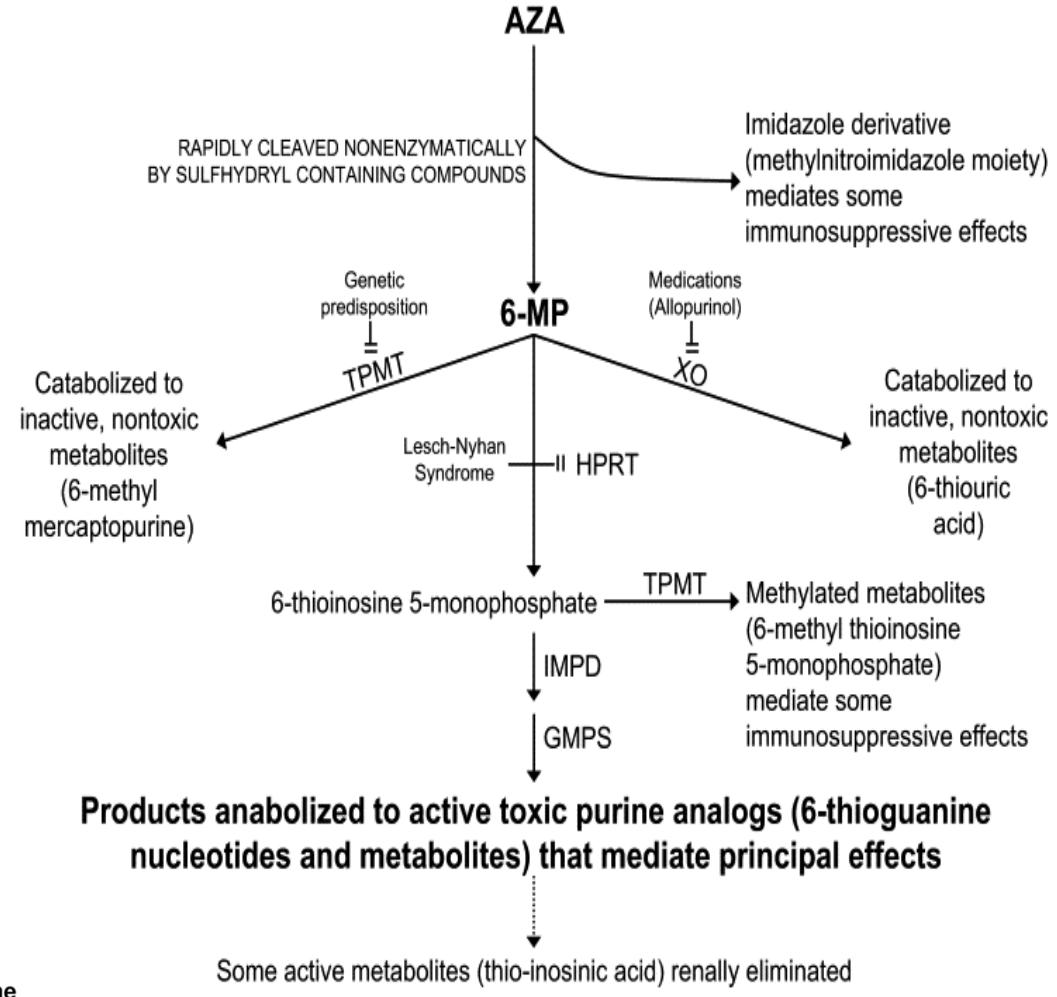
- It is a prescription medicine used to treat the symptoms of Rheumatoid Arthritis and as prevention of transplant rejection.



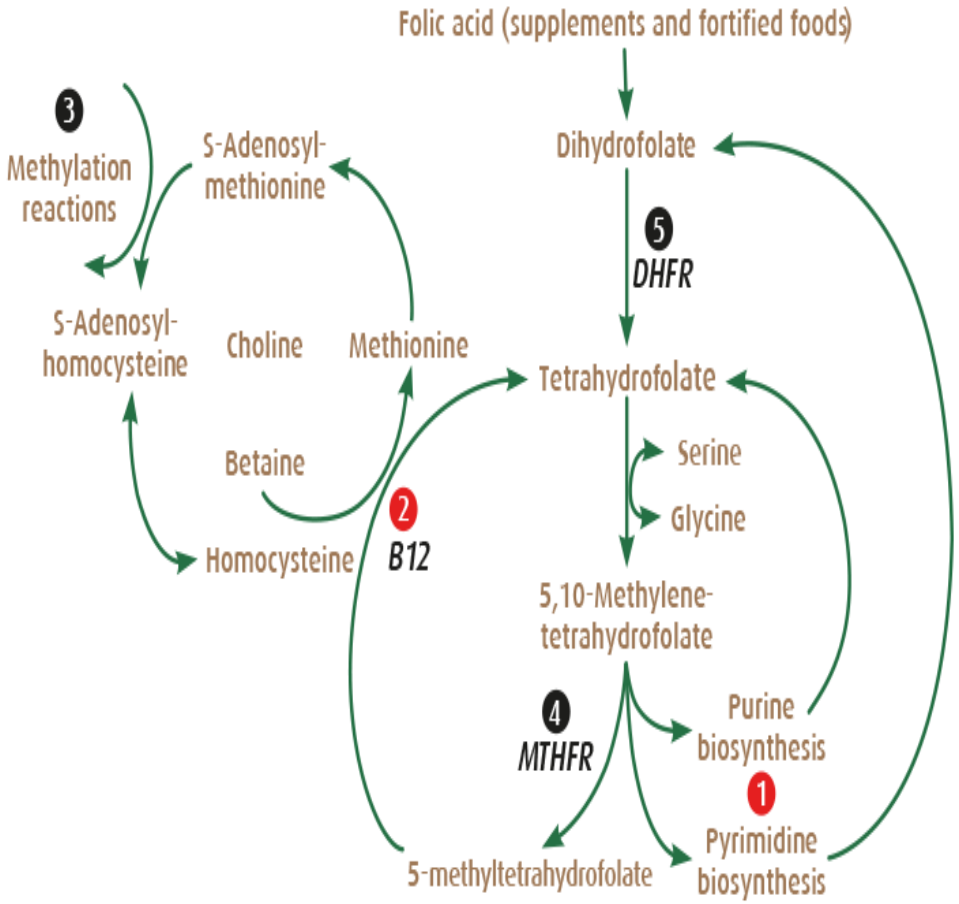
Azathioprine (prodrug)

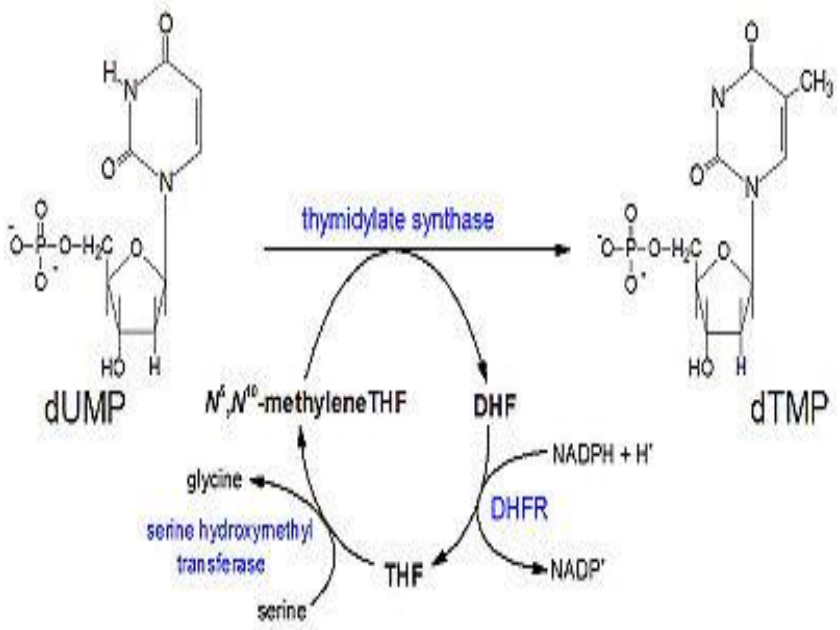


6-Mercaptopurine



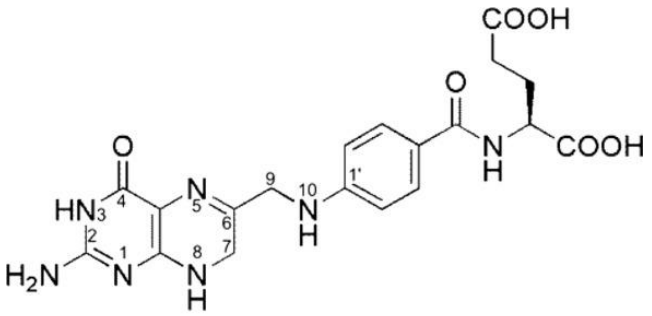
DİHYDROFOLATE REDUCTASE (DHFR) MECHANISM





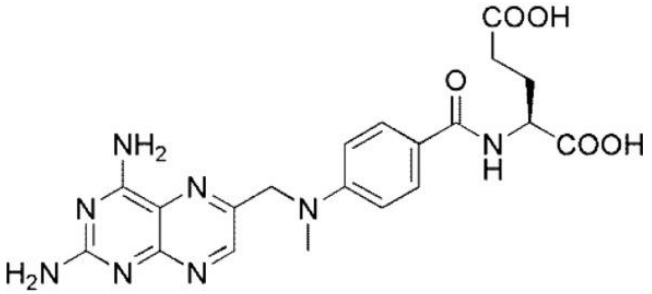
Methotrexate

- **Dihydrofolate reductase inhibitors are an important class of drugs, as evidenced by their use as antibacterial, antimalarial, antifungal, and anticancer agents**. Progress in understanding the biochemical basis of mechanisms responsible for enzyme selectivity and antiproliferative effects has renewed the interest in antifolates for cancer chemotherapy and prompted the medicinal chemistry community to develop novel and selective human DHFR inhibitors, thus leading to a new generation of DHFR inhibitors



Dihydrofolate (DHF)

- Methotrexate interferes with the growth of certain cells of the body, especially cells that reproduce quickly, such as cancer cells, bone marrow cells, and skin cells.

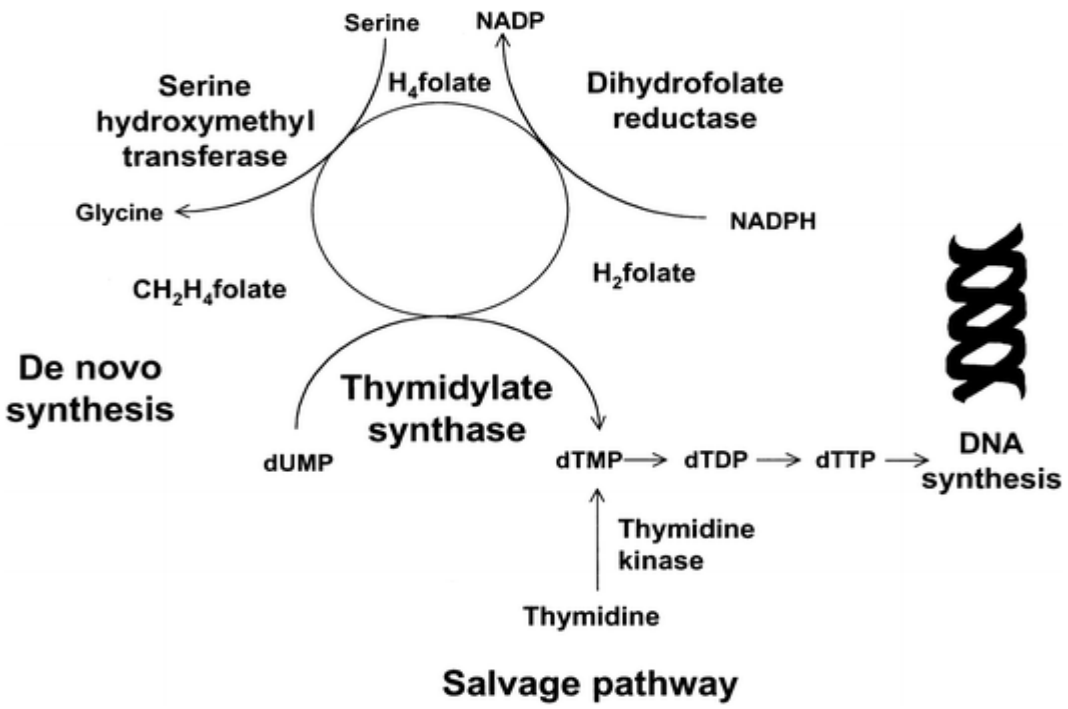


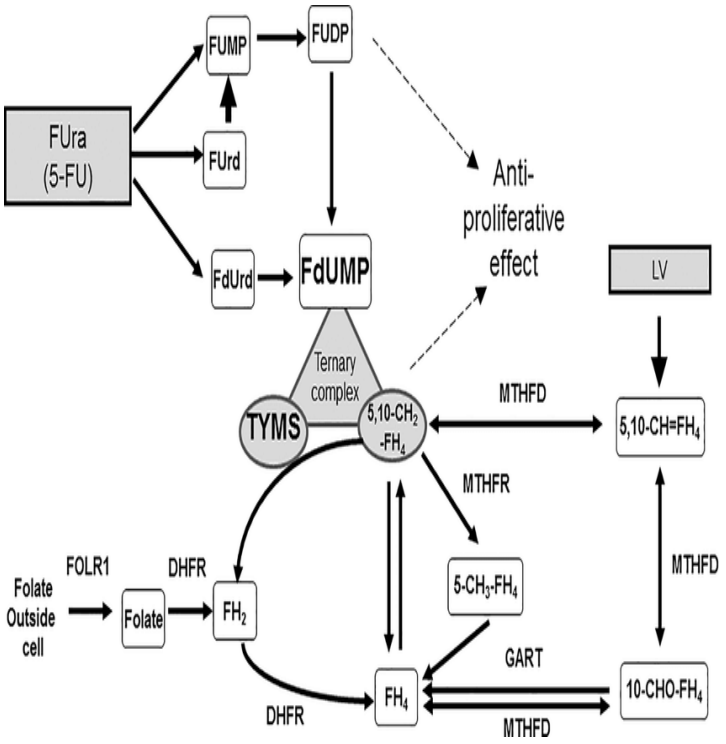
Methotrexate (MTX)

- Methotrexate is used to **treat leukemia and certain types of cancer of the breast, skin, head and neck, lung, or uterus.**

Methotrexate is also used to treat **severe psoriasis and rheumatoid arthritis in adults. I**t is also used to treat active polyarticular-course juvenile rheumatoid arthritis in children.

5-fluorouracil (5-FU)





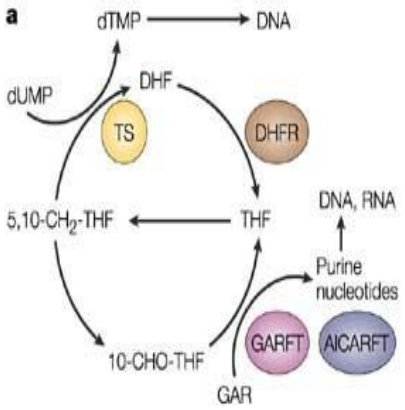
The fluoropyrimidine 5-fluorouracil (5-FU) is an antimetabolite drug that is widely used for the treatment of cancer, particularly for colorectal cancer.

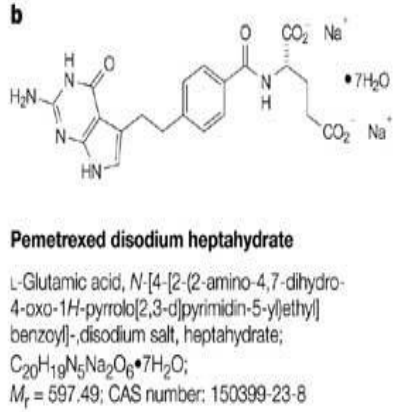
5-FU exerts its anticancer effects through inhibition **of thymidylate synthase (TS) and incorporation of its metabolites into RNA and DNA.**

Modulation strategies, such as co-treatment with methotrexate, have been developed to increase the anticancer activity of 5-FU.

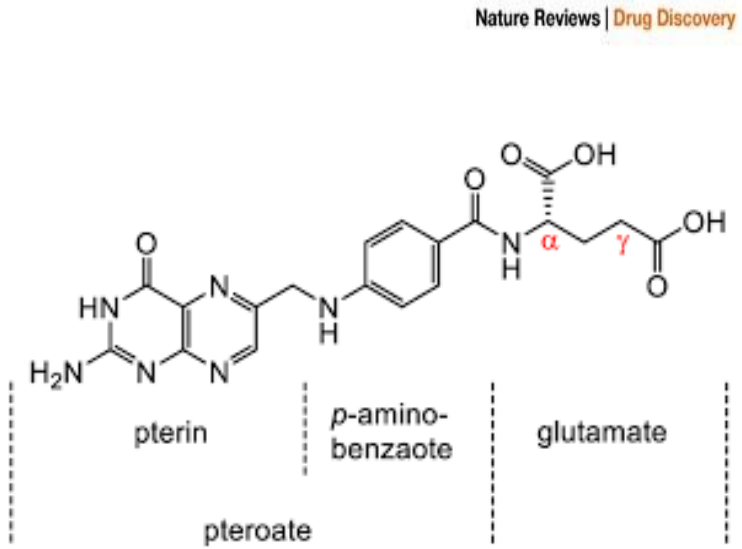
Pemetrexed

- Pemetrexed is a multitarget antifolate that inhibits folate-dependent enzymes: **thymidylate synthase, dihydrofolate reductase and glycinamide ribonucleotide formyltransferase,** required for de novo synthesis of nucleotides for DNA replication.



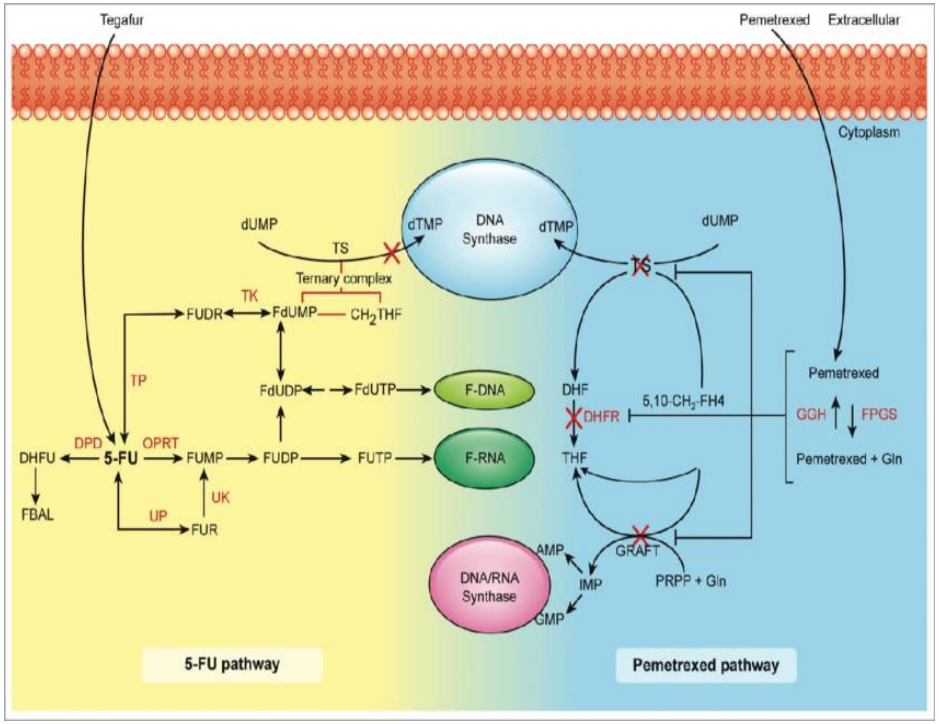


- It is currently used in the treatment of **mesothelioma and non-small cell lung cancer (NSCLC),** and has shown clinical activity in other tumors such as breast, colorectal, bladder, cervical, gastric and pancreatic cancer.



Capecitabine

- Capecitabine used in the treatment of metastatic breast and colorectal cancers.
- **Capecitabine is a prodrug, that is enzymatically converted to fluorouracil (antimetabolite) in the tumor**, where it inhibits DNA synthesis and slows growth of tumor tissue.
- For the treatment of patients with **metastatic breast cancer resistant to both paclitaxel and an anthracycline-containing chemotherapy regimen.**



- Capecitabine is used alone as an adjuvant therapy following the complete resection of primary tumor in patients with **stage III colon cancer when monotherapy with fluroprymidine is preferred.** The use or capecitabine in combination regimens for advanced gastric cancer is currently being investigated.

Pentostatin (2'-deoxycoformycin; dCF)

A potent inhibitor of adenosine deaminase. The drug is effective in the treatment of many lymphoproliferative malignancies, particularly hairy-cell leukemia. It is also synergistic with some other antineoplastic agents and has immunosuppressive activity. **Pentostatin (2'-deoxycoformycin; dCF) and cladribine (2-chlorodeoxyadenosine; CdA) are highly effective agents for the treatment of hairy cell leukemia**.

dCF is a potent inhibitor of adenosine deaminase (ADA), and treatment results in the accumulation of deoxyadenosine (dAdo) and adenosine (Ado) in the plasma. **dAdo is phosphorylated by deoxycytidine kinase in lymphocytes to deoxyadenosine monophosphate (dAMP**), which is subsequently converted to deoxyadenosine triphosphate (**dATP). Both dATP and CdATP cause an initial accumulation of DNA strand breaks in lymphocytes** and this **results in the activation of p53, the release of cytochrome c from mitochondria, and apoptosis.**

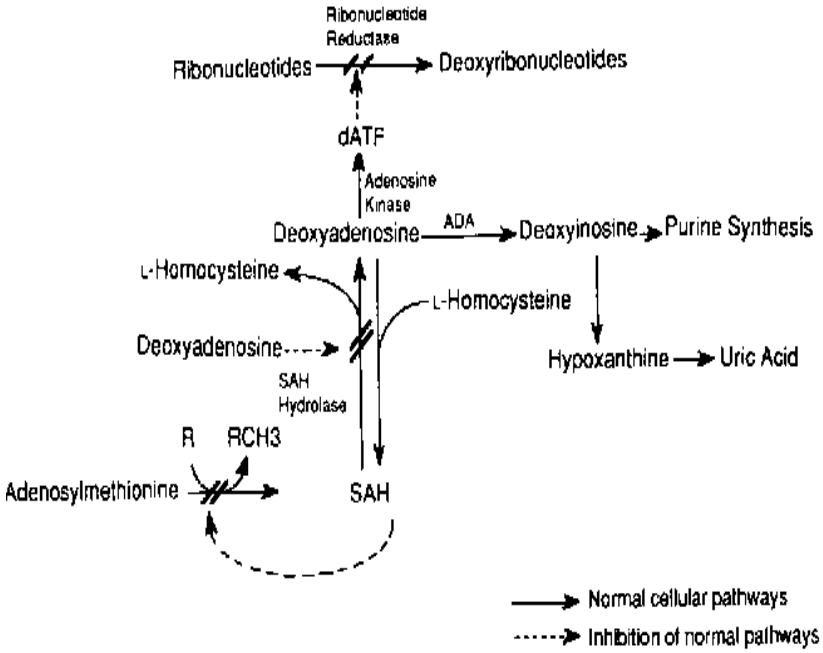
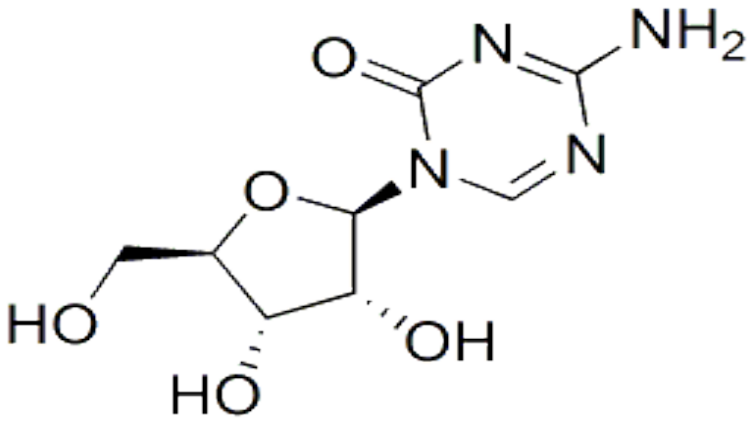
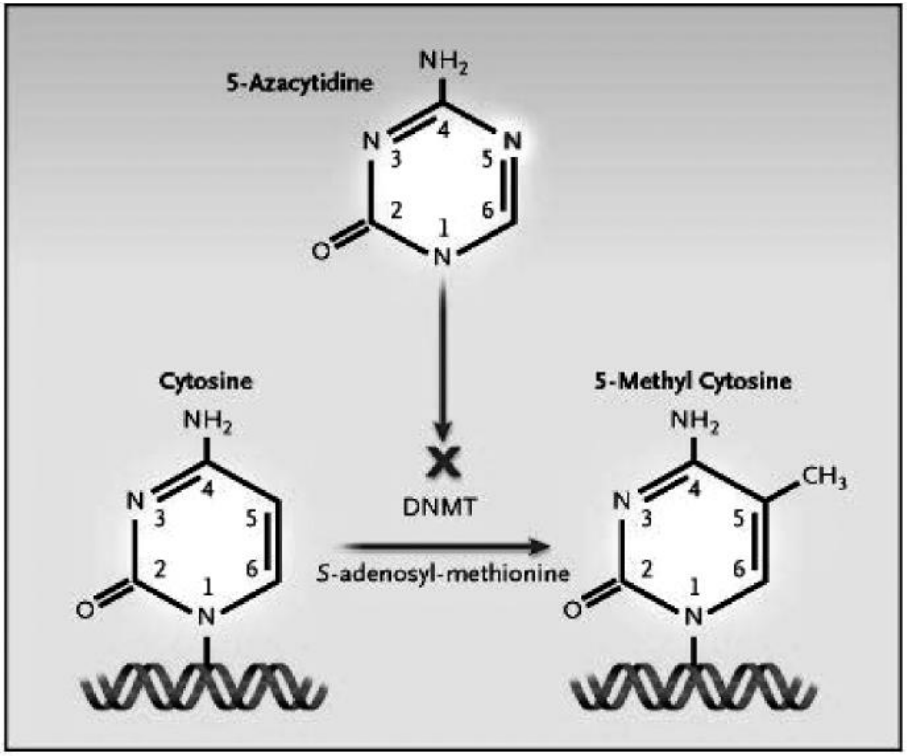


Figure 2. Pathways of intracellular deoxyadenosine use and degradation. ADA = adenosine deaminase; dATP = deoxyadenosine triphosphate; R = parent compound; SAH = *S*-adenosyl-L-homocysteine.

Azacitidine,

- Azacitidine, is used mainly in the treatment of myelodysplastic syndromeAzacitidine (5-azacytidine) is a chemical analogue of the cytosine nucleoside used in DNA and RNA.
- Azacitidine may induce antineoplastic activity by inhibition of **DNA methyltransferase** at low doses and cytotoxicity through incorporation into RNA and DNA at high doses. The finding that 5-azacytidine was incorporated into DNA and that, when present in DNA, it inhibited DNA methylation, led to widespread use of 5-azacytidine and 5-aza-2′-deoxycytidine (Decitabine) to demonstrate the correlation between loss of methylation in specific gene regions and activation of the associated genes.



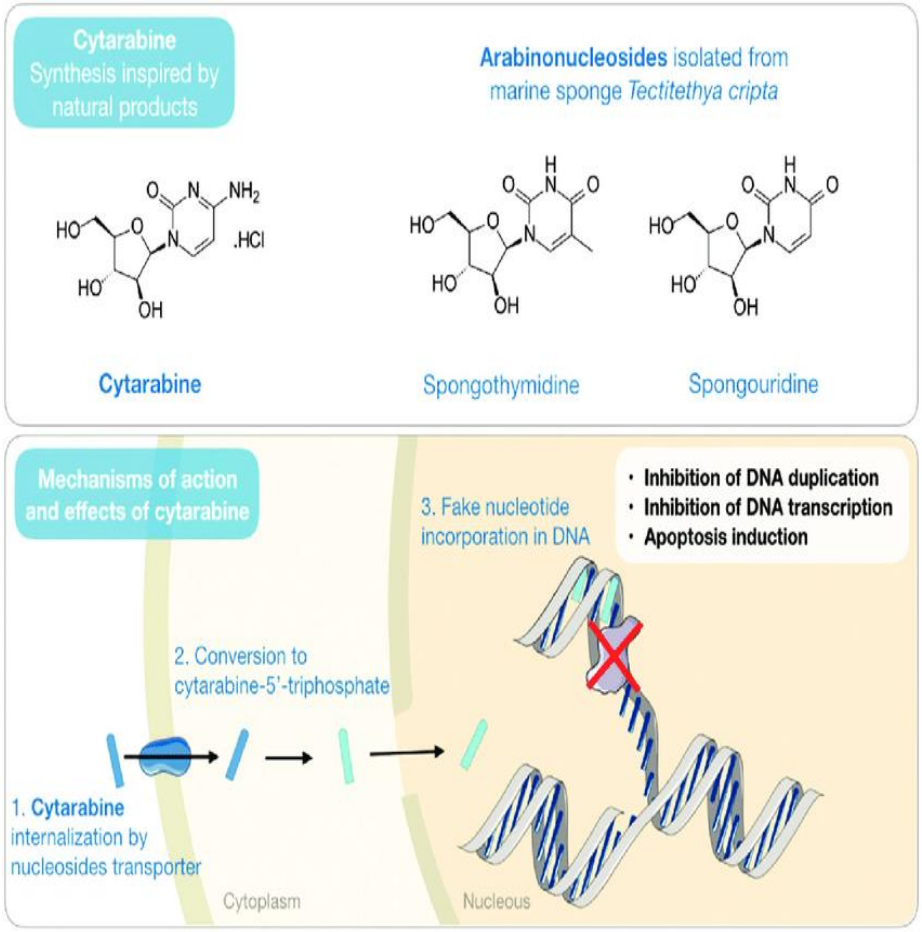


- There is now a revived interest in the use of Decitabine as a therapeutic agent for cancers in which epigenetic silencing of critical regulatory genes has occurred. Here, the current status of our understanding of the mechanism(s) by which 5-azacytosine residues in DNA inhibit DNA methylation is reviewed with an emphasis on the interactions of these residues with bacterial and mammalian DNA (cytosine-C5) methyltransferases. The implications of these mechanistic studies for development of less toxic inhibitors of DNA methylation are discussed.

Cytarabine (cytosine arabinoside)

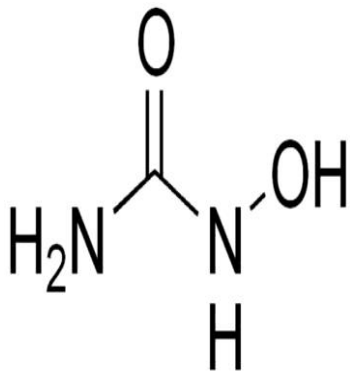
- It is an analogue **of 2'-deoxycytidine that inhibits DNA polymerase activity** after being incorporated into DNA as a fraudulent nucleotide, **resulting in impaired DNA synthesis.**

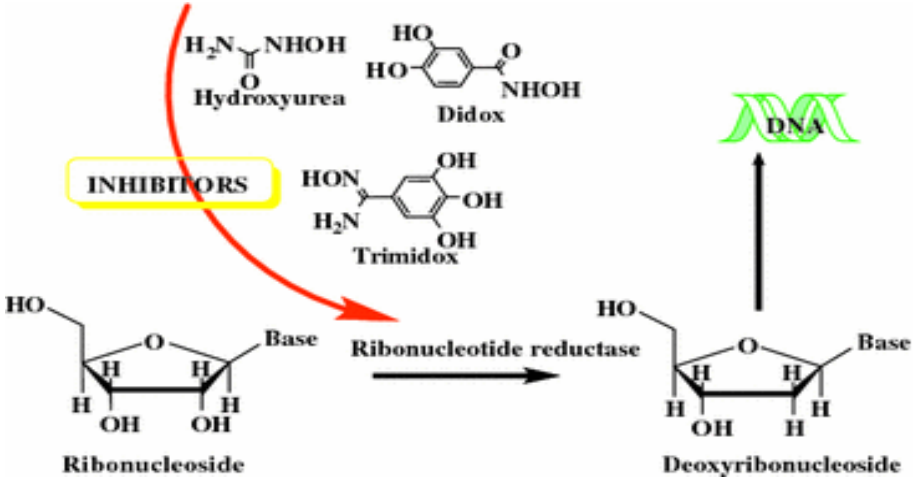
- Cytarabine is a pyrimidine nucleoside analogue used to treat acute non-lymphocytic leukemia, lymphocytic leukemia, and the blast phase of chronic myelocytic leukemia.

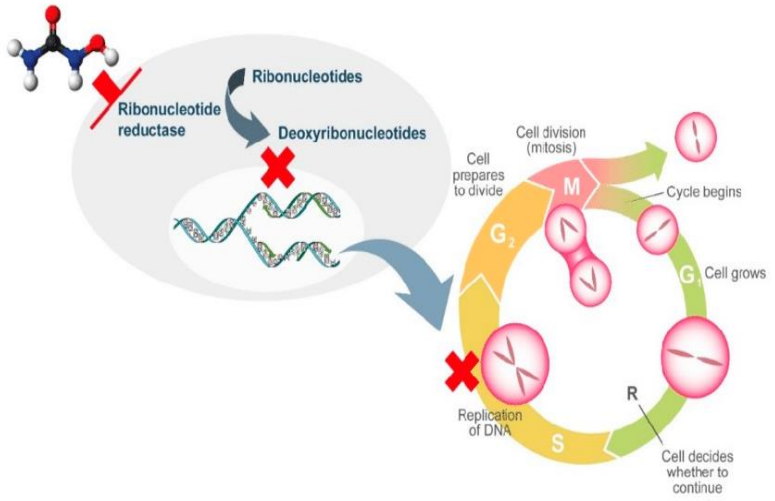


Hydroxyurea

- **It is well absorbed after oral administration, converted to a free radical nitroxide in vivo, and transported by diffusion into cells where it quenches the tyrosyl free radical at the active site of the M2 protein subunit of ribonucleotide reductase, inactivating the enzyme.**
- **The entire replitase complex, including ribonucleotide reductase, is inactivated and DNA synthesis** is selectively inhibited**, producing cell death in S phase and synchronization of the fraction of cells that survive**.
- Repair of DNA damaged by chemicals or irradiation is also inhibited by hydroxyurea, **offering potential synergy between hydroxyurea and radiation or alkylating agents.**
- . Hydroxyurea is used to treat cancer of the white blood cells called chronic myeloid leukemia (CML). It may also be given together with radiation treatment for head and neck cancer (advanced squamous cell cancer).
- Hydroxyurea is also used in adult patients with sickle cell anemia to prevent painful episodes and reduce the need for blood transfusions.





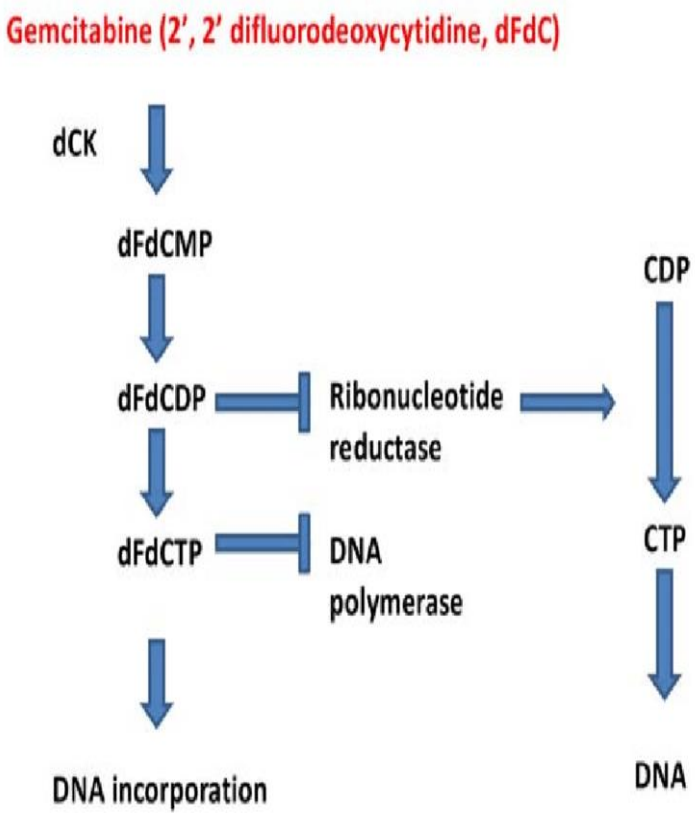


Gemsitabin (dFdC),

- **Gemcitabine (dFdC) is a new anticancer nucleoside that is an analog of deoxycytidine**. It is a pro-drug and, once transported into the cell, **must be phosphorylated by deoxycytidine kinase to an active form.**
- **Both gemcitabine diphosphate (dFdCTP) and gemcitabine triphosphate (dFdCTP**) **inhibit processes required for DNA synthesis**. **Incorporation of dFdCTP into DNA** is most likely the major mechanism by which gemcitabine causes cell death.

- **After incorporation of gemcitabine nucleotide on the end of the elongating DNA strand,** one more deoxynucleotide is added and thereafter, the DNA polymerases are unable to proceed. This action ("masked termination") apparently locks the drug into DNA as the proofreading enzymes are unable to remove gemcitabine from this position.

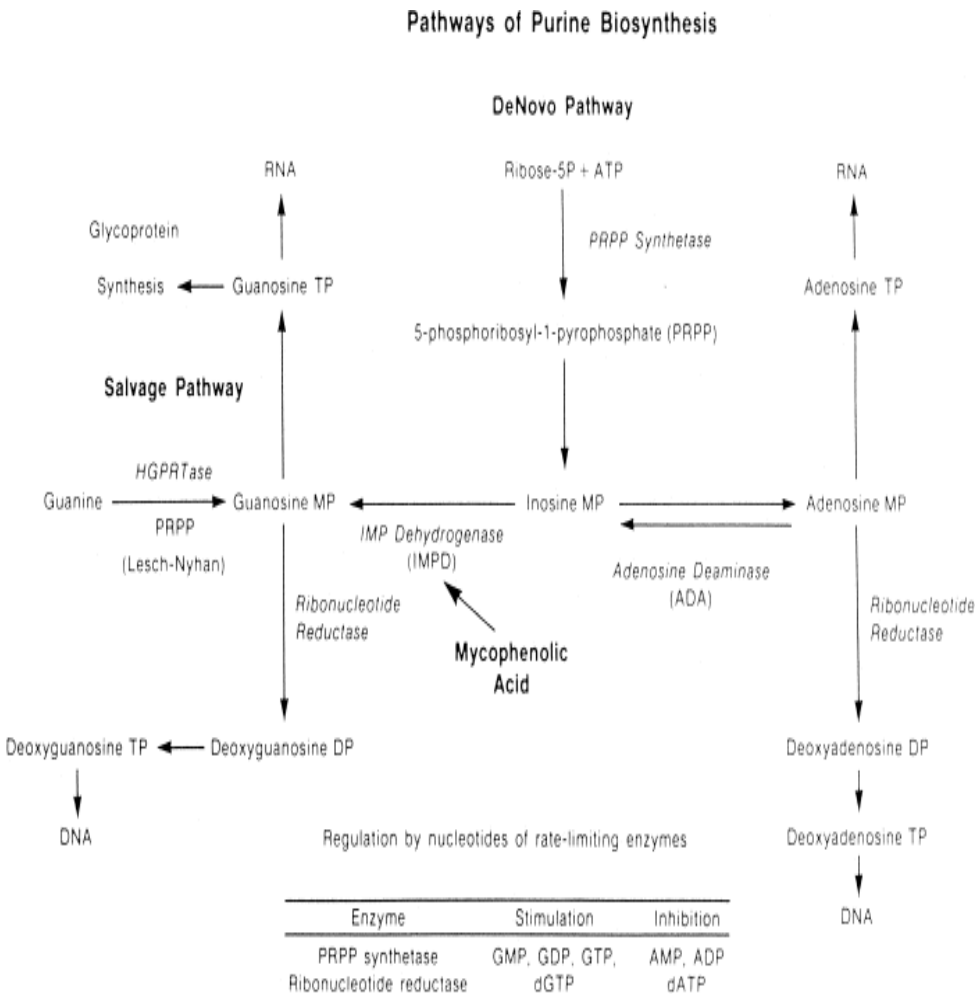
- Gemcitabine is used to treat certain types of cancer (including breast, lung, ovarian, pancreatic).
- It is a chemotherapy drug that works by slowing or stopping the growth of cancer cells.



Mycophenolate mofetil (MMF, CellCept)

- **It is a prodrug of mycophenolic acid (MPA), an inhibitor of inosine-5'-monophosphate dehydrogenase.** MPA depletes guanosine nucleotides preferentially in T and B lymphocytes and inhibits their proliferation, thereby suppressing cell-mediated immune responses and antibody formation.
- **MPA also inhibits the glycosylation and expression of adhesion molecules, and the recruitment of lymphocytes and monocytes into sites of inflammation.**
- **MPA depletes tetrahydrobiopterin**
- and **decreases the production of nitric oxide by inducible NO synthase without affecting the activity of constitutive NO synthases.**

- Mycophenolate has been used to treat people with lupus (especially those with symptoms of **kidney disease),** Rheumatoid arthritis (RA), vasculitis, inflammatory bowel disease such as **Crohn's disease,** inflammatory eye disease (such as **uveitis (iritis) and scleritis),** and some other kidney and skin disorders.



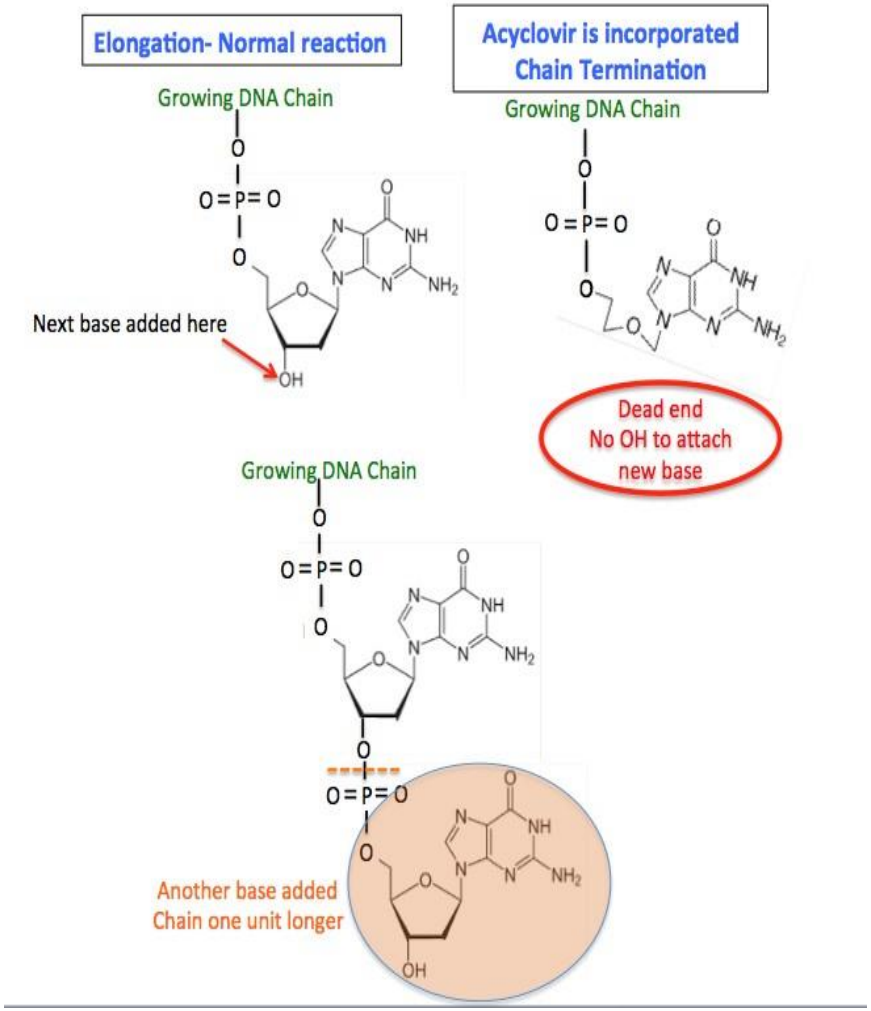
Acyclovir,

- **Acyclovir, an acyclic purine nucleoside analog, is a highly potent inhibitor of herpes simplex virus (HSV), types 1 and 2, and varicella zoster virus, and has extremely low toxicity for the normal host cells**.

- This selectivity is due to the ability of these viruses to code for a viral **thymidine kinase capable of phosphorylating acyclovir to a monophosphate, this capability is essentially absent in uninfected cells.**
- The acyclovir monophosphate (acyclo-GMP) is subsequently **converted to acyclovir triphosphate (acyclo-GTP) by cellular enzymes.**

- **The amounts of acyclo-GTP formed in HSV-infected cells are 40 to 100 times greater than in uninfected cells**.
- Acyclo-GTP acts as a more potent inhibitor of the **viral DNA polymerases than of the cellular polymerases.**

- **The DNA polymerases of HSV-1 and HSV-2 also use acyclo-GTP** as a substrate and incorporate acycto-GMP into the DNA primer-template to a much greater extent than do the cellular enzymes, **the viral DNA polymerase binds strongly to the acyclo-GMP-terminated template, and is thereby inactivated**



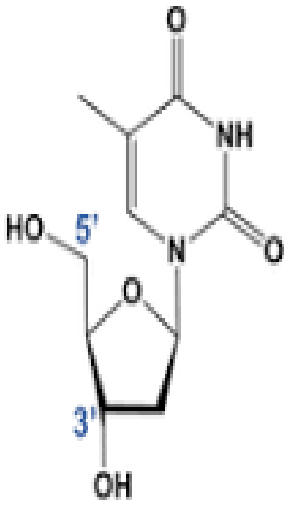
Azidothymidine

. Azidothymidine is a analog of thymidine

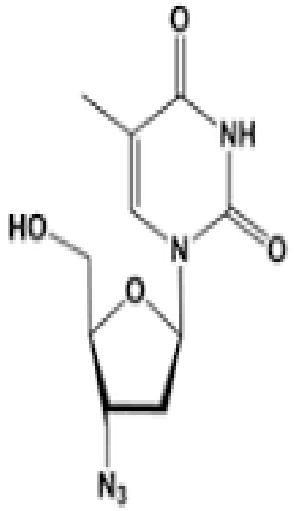
- **Azidothymidine used for the treatment or prevention of HIV/AIDS.**
- Azidothymidine used to **prevent the spread of HIV/AIDS from mother-to-child during birth** or after a needlestick injury or other potential exposure.

- Azidothymidine or zidovudine is a prodrug which phosphorylate its active 5′-triphosphate metabolite and form a zidovudine triphosphate (ZDV-TP).

- Zidovudine triphosphate (ZDV-TP) competes with endogenous nucleotides for **incorporation into the viral DNA.**
- And, Once it incorporated it will **initiate the chain termination due to the lack of a 3' OH group**



Thymidine



Azidothymidine (AZT)
Zidovudine

Ganciclovir

Ganciclovir is an oxopurine that is **guanine substituted by a [(1,3-dihydroxypropan-2-yl)oxy]methyl group at position 9.**
It has a role as an antiviral drug and an antiinfective agent. **Ganciclovir used for the treatment of cytomegalovirus (CMV) infections. This drug also has activity against nearly all herpesviruses human papillomavirus, or RNA viruses such as influenza A.** The antiviral activity of Ganciclovir helps to inhibit virus replication.

Thymidine kinase catalyzes the phosphorylation of ganciclovir to the monophosphate. Then this monophosphate subsequently converted into **the Ganciclovir diphosphate with the help of a cellular enzyme, guanylate kinase.**
Then Ganciclovir diphosphate converted into **Ganciclovir triphosphate by a number of cellular enzymes.**
Next, this newly formed Ganciclovir triphosphate competitively **inhibits the incorporation of deoxyguanosine triphosphate into elongating DNA and leading to the formation of 'faulty' DNA.**
Now ganciclovir triphosphate is started to incorporate into the DNA strand by **replacing many of the adenosine bases.** This results in the prevention of DNA synthesis, as phosphodiester bridges can longer be built, destabilizing the strand.

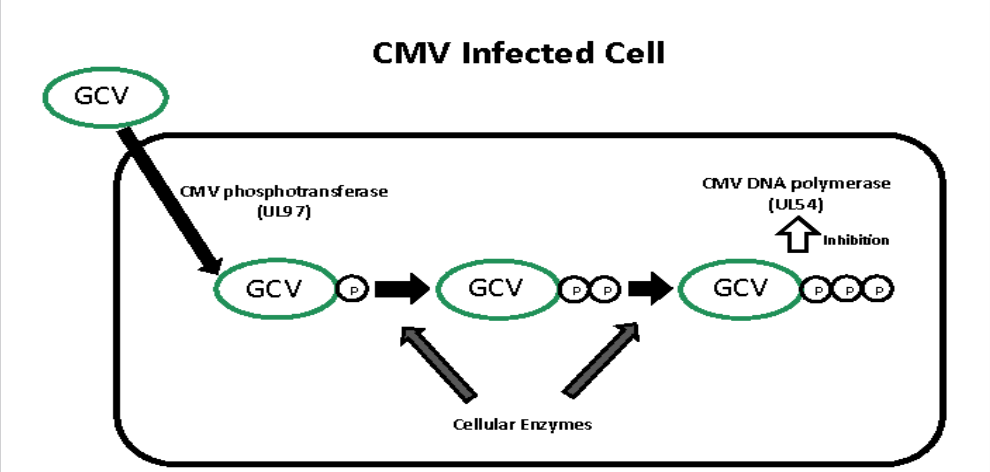
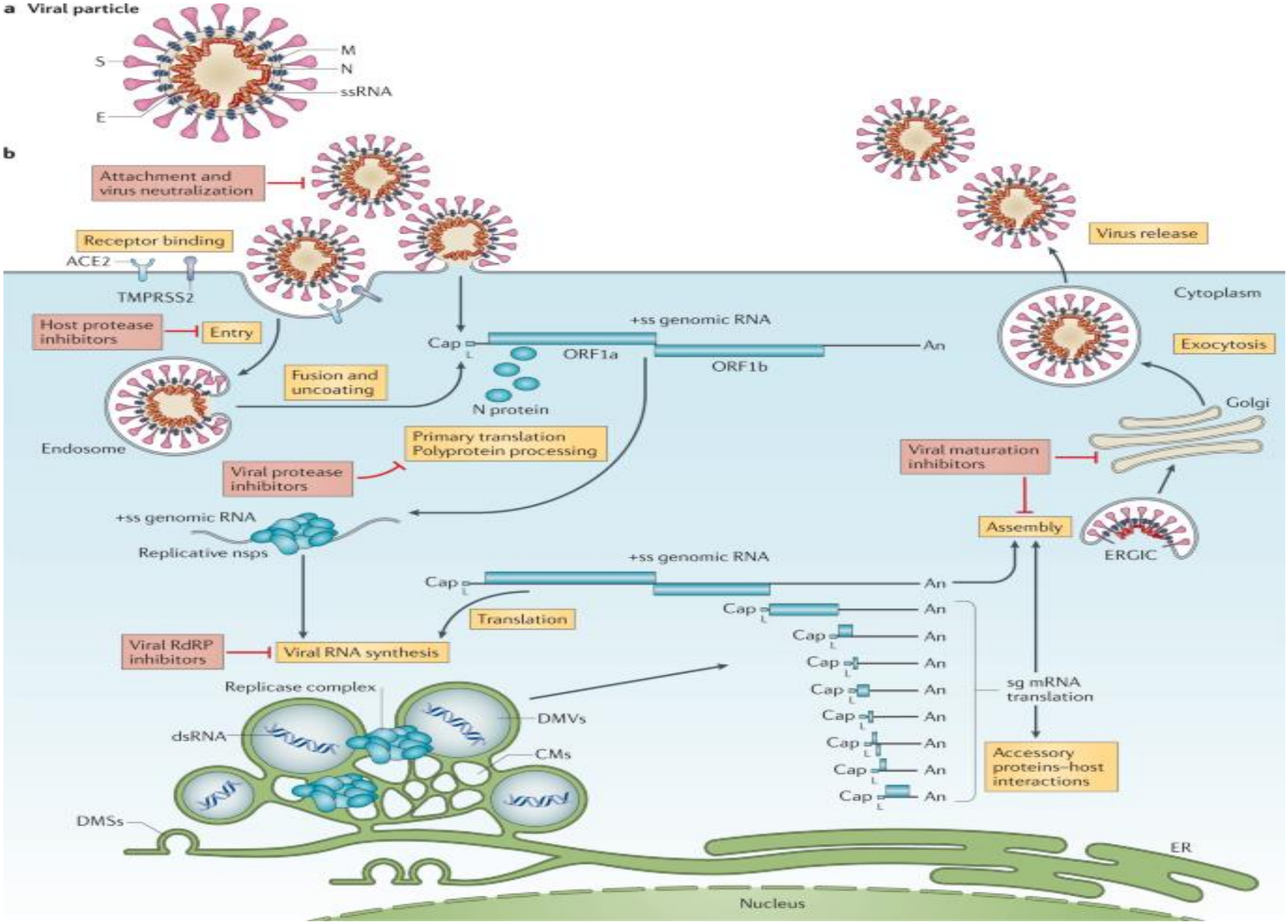
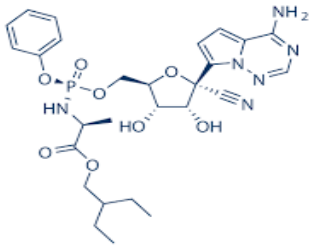


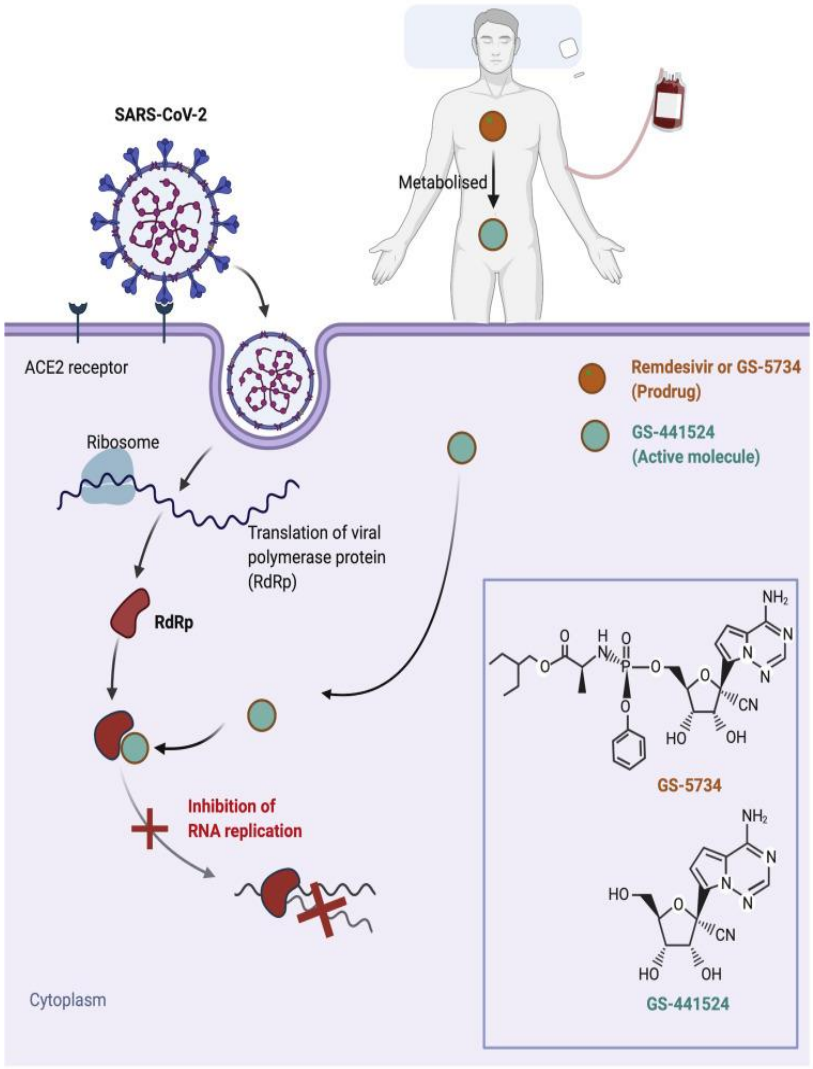
Figure 1: Mechanism of action of Ganciclovir in CMV infection.


a Viral particle
S
M
N
ssRNA
E
b
Attachment and virus neutralization
Receptor binding
ACE2
TMPRSS2
Host protease inhibitors
Entry
Fusion and uncoating
Endosome
Cap
ORF1a
ORF1b
An
+ss genomic RNA
N protein
Primary translation Polyprotein processing
Viral protease inhibitors
+ss genomic RNA
Replicative nsps
Viral RdRP inhibitors
Viral RNA synthesis
Translation
Replicase complex
dsRNA
DMVs
CMs
DMSs
sg mRNA translation
Accessory proteins–host interactions
Assembly
ERGIC
Golgi
Viral maturation inhibitors
Virus release
Cytoplasm
Exocytosis
ER
Nucleus

Remdesivir (RDV)

- **The newest and most important work on the antimetabolite theory is on remdesivir (RDV).**
- RDV is a phosphoramidate prodrug of a 1-cyano-substituted nucleotide analogue
- **<u>Its triphosphate form (RDV-TP) resembles ATP</u> and is used as a substrate of several viral RNA dependent RNA polymerase (RdRp) enzymes or complexes.**
- Remdesivir, being a prodrug, is metabolized into its active form competes **with ATP for incorporation into RNA and inhibits the action of viral RNA-dependent RNA polymerase.**
- **This results in the termination of RNA transcription and decreases viral RNA production. Remdesivir is authorized by FDA for the treatment of hospitalized patients with severe COVID-19 disease on 1 May 2020.**





NEW GOALS

PRPP SYNTHETASE ; Phosphoribosylpyrophosphate(PRPP) synthetase(PRS) catalyzes the formation of PRPP from ATP and ribose-5-phosphate. PRPP is an important substrate for the synthesis of purine, pyrimidine, and pyridine dinucleotides.

HYPOXANTHİNE GUANİNE TRANSFERASE (HGPRT)- It is the key enzyme for the HPRT purine salvage pathway that uses hypoxanthine and guanine as substrates. The enzyme catalyzes the conversion of guanine and hypoxanthine to the respective nucleoside monophosphates, by using PRPP as donor of the phosphoribosyl moiety.

THYMİDİNE KİNASE (TK) ; TK1 converts the deoxythymidine (dT) nucleoside to deoxythymidine monophosphate (dTMP). It takes the phosphate group from ATP enzymatically. **Thymidine kinase enzyme can be used for diagnosis and prognosis in cancer patients.his enzyme, of which the molecular structure has been clarified, can be a key enzyme in the discovery of new active metabolites.**

5 'NUCLEOTİDASE (5'NT) ; 5' nucleosidase, one of the key enzymes of the purine pathway, in addition to its phosphatase activity contrary to what is believed, diversity of substrates and particularly use of synthetic pyrimidines as substrate provide investigators with important possibilities. **5 'NT has kinase and phosphatase activity in the organism and this feature makes the enzyme special.This shows us the importance of studies on enzymes and enzyme kinetics.**

CİTİDİNE DEAMİNASE (CD) : Cytidine deaminase deaminate cytidine to uridine play an important role in a variety of pathways from bacteria to man. Ancestral members of this family were able to deaminate cytidine only in a mononucleotide or nucleoside context. **This enzyme plays a key role particularly in the catabolic pathway of purines. Enzymes that Recently, a family of enzymes has been discovered with the ability to deaminate cytidines on RNA or DNA**

Anti Metabolite Theory

- Antimetabolite theory is based on a very simple rationale. While the simplicity of the rationale has remained valid starting from the introduction of the theory, limitations of this field direct the investigators to more popular areas with different reasons. **The rate of development of the knowledge on nucleic acids and studies on the application of this knowledge to technology have overshadowed especially this important field.**

- **Key enzymes that carry basic information in nucleic acid and purine-pyrimidine metabolism are at the center of the antimetabolite theory**. Increasing our knowledge of enzymes will not only pave the way for the use of new antimetabolites in this field, but also improve our knowledge of other important metabolic functions, particularly nucleic acid and nucleotide metabolism.

- **Generation of synthetic proteins by bioengineering, discovery of antimetabolites that target membrane receptors of cancer cells, synthesis of key enzyme inhibitors through protein-protein interactions could lead to new developments. If we improve our knowledge of the kinetics of key enzymes of nucleotide and nucleic acid metabolism,**

- **We will be able to make significant advances in this area. Although there is a long road ahead, antimetabolite theory will guide us in this area.**