Antimetabolites

- Anti-metabolites masquerade as purine or pyrimidine - which become the building blocks of DNA.
- They prevent these substances becoming incorporated in to DNA during the "S" phase (of the cell cycle), stopping normal development and division.

Antimetabolites

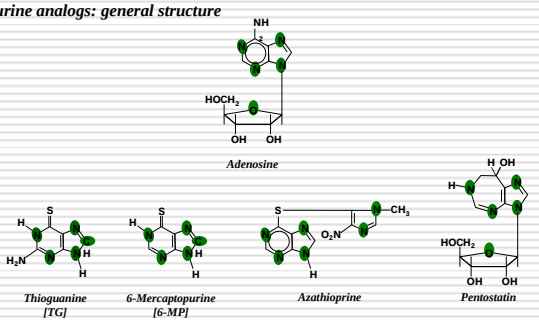
- Mimic structures of normal metabolic molecules
  - Inhibit enzymes competitively OR
  - Incorporation into macromolecules
    - inappropriate structures
- Kill cells in S phase
- Three main groups
  - Purine analogs
  - Pyrimidine analogs
  - Folate antagonists

Antimetabolites

Class of antimetabolite	*Drugs in class*	*Disease*
1. Purine analogs	***Mercaptopurine [6-MP]***	*Leu*
	Thioguanine [TG]	*Leu*
	Pentostatin	*Leu, Lym*
	Cladribine	*Leu, Lym*
	Fludarabine	*Leu, Lym*
2. Pyrimidine analogs	***Fluorouracil [5-FU]***	*Solid*
	Floxuridine	*Solid*
	Cytarabine [AraC]	*Leu*
	Gemcitabine	*Solid*
	5-Azacytidine	*Solid*
3. Folate analogs	***Methotrexate*** *[Amethopterin, MTX]*	*Leu, Solid, Sar*
	Permetrexed [experimental]	

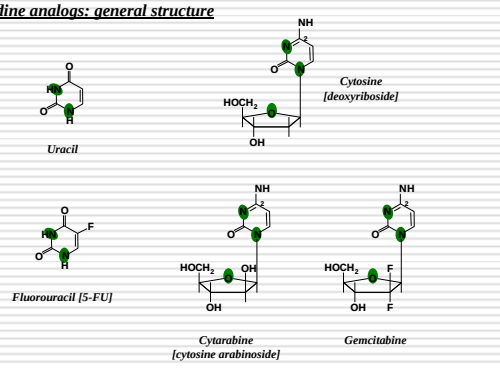
Antimetabolites

Purine analogs: general structure



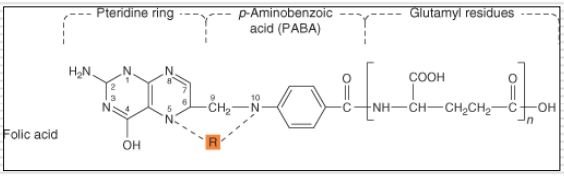
Antimetabolites

Pyrimidine analogs: general structure



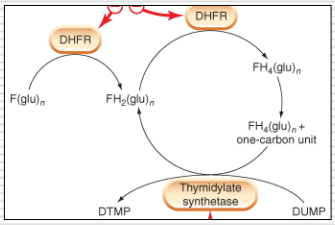
Folic Acid

- Folic acid is needed for the *de novo* synthesis of the nucleoside thymidine, required for DNA synthesis.
- Also, folate is needed for purine base synthesis
- Folate: pteridine ring + PABA + glutamate
  - In cells, converted to polyglutamates then → tetrahydrofolate (FH_4)

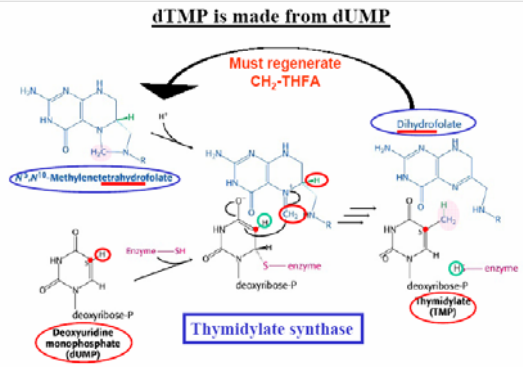


Folic Acid

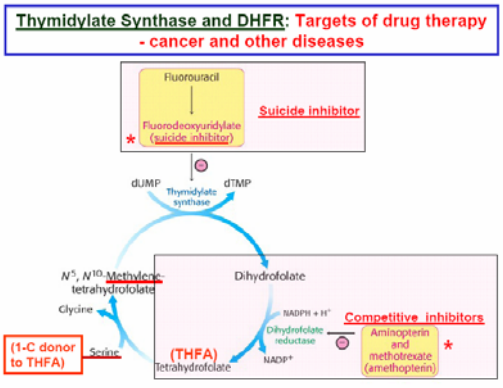
- Folate → FH4 catalysed by dihydrofolate reductase in 2 steps:
  - Folate → FH2
  - FH2 → FH4
- FH4 serves as methyl group donor (1-C unit) to deoxyuridine (dUMP → dTMP), also regenerating FH2

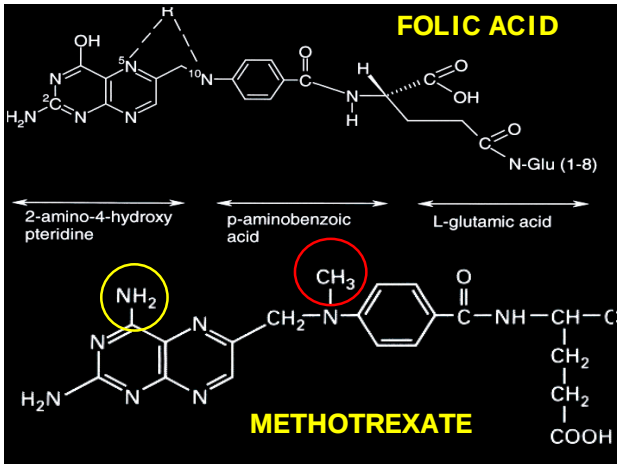


Folic Acid



Folic Acid





Folic Acid

- Higher affinity for enzyme than does FH2
  - Additional H or ionic bond forms
  - → Depletion FH4 in cell → depleting dTMP → "thymine-less death"
  - → Inhibiting DNA synthesis
- Uptake through folate transport system
  - Resistance through decreased uptake
- Metabolites (polyglutamate derivatives) retained for weeks, months

Methotrexate

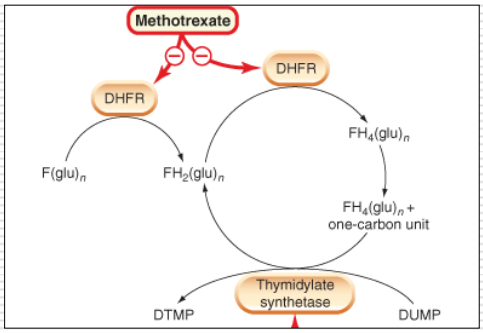
Mode of Action

- Methotrexate acts specifically during DNA and RNA synthesis, and thus it is cytotoxic during the S-phase of the cell cycle
- Binds to dihydrofolate reductase which leads to reduction of tetrahydrofolate, which inhibits pyrimidine synthesis.
  - Pyrimidine is needed for formation of DNA base pairs, therefore decrease in DNA replication esp rapidly dividing cells as in skin
- Induces apoptosis of activated T cells

Methotrexate

- Methotrexate inhibits folic acid reductase which is responsible for the conversion of folic acid to tetrahydrofolic acid.
- At two stages in the biosynthesis of purines (adenine and guanine) and at one stage in the synthesis of pyrimidines (thymine, cytosine, and uracil), one-carbon transfer reactions occur which require specific coenzymes synthesized in the cell from tetrahydrofolic acid.
- Tetrahydrofolic acid itself is synthesized in the cell from folic acid with the help of an enzyme, folic acid reductase.
- Methotrexate looks a lot like folic acid to the enzyme, so it binds to it thinking that it is folic acid. In fact, methotrexate looks so good to the enzyme that it binds to it quite strongly and inhibits the enzyme.
- Thus, DNA synthesis cannot proceed because the coenzymes needed for one-carbon transfer reactions are not produced from tetrahydrofolic acid because there is no tetrahydrofolic acid. Again, without DNA, no cell division.

Methotrexate

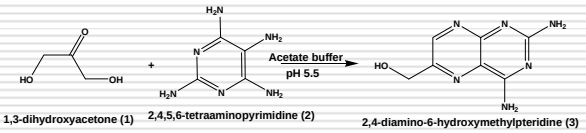


Methotrexate

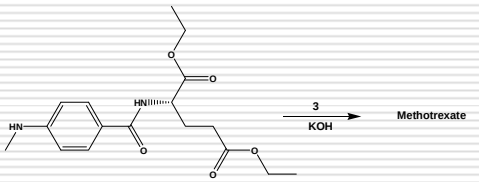
Dosage

- Initial: 2.5-5mg q12h x3 doses qweek
- Titrate up weekly by 2.5mg increments [if blood counts (weekly then monthly) and LFTs (q4 month)allow] until symptoms respond
- Injections: IM or SQ
  - Max: 50mg/week, but some 75mg/week

Synthesis of Methotrexate

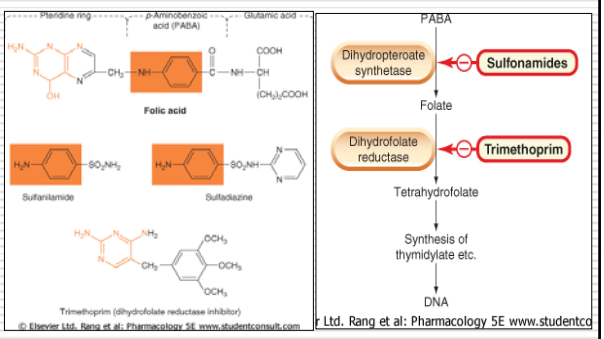


1,3-dihydroxyacetone (1) 2,4,5,6-tetraaminopyrimidine (2) 2,4-diamino-6-hydroxymethylpteridine (3)

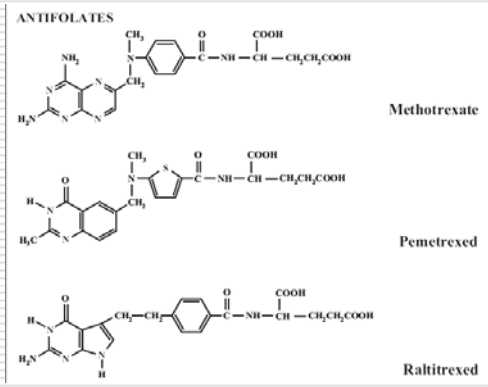


diethyl N-[4-(methylamino)benzoyl]-L-glutamate (4)

Folate Antagonists

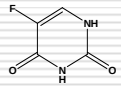


Folate Antagonists



5-Fluorouracil

- Chemical Formula $C_4H_3FN_2O_2$
- Trade Name Efudex, Fluoroplex
- Dosage Forms
  - Solution Intravenous
  - Cream Topical



5-Fluorouracil

5-Fluorouracil

- It blocks an enzyme which converts the cytosine nucleotide into the deoxy derivative.
- Converted → "fraudulent" nucleotide FdUMP
- In addition, DNA synthesis is further inhibited because Fluorouracil blocks the incorporation of the thymidine nucleotide into the DNA strand
- Fluorouracil inhibits thymidylate synthetase, leading to inhibition of DNA and RNA synthesis and cell death.
  - Competitive inhibitor for thymidylate synthetase active site, but can't be converted to dTMP
- Mechanism of action uses all 3routes → decreased DNA synthesis, also transcription/translation inhibition

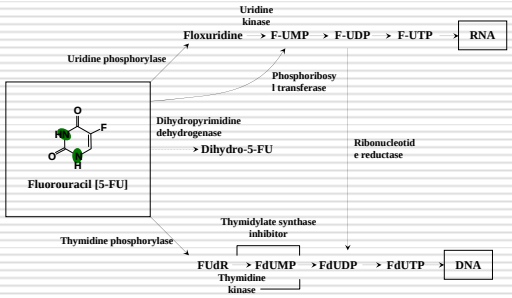
5-Fluorouracil

Mode of action

- As a pyrimidine analogue, it is transformed inside the cell into different cytotoxic metabolites which are then incorporated into DNA and RNA, finally inducing cell cycle arrest and apoptosis by inhibiting the cell's ability to synthesize DNA.
- It is an S-phase specific drug and only active during certain cell cycles.
- In addition to being incorporated in DNA and RNA, the drug has been shown to inhibit the activity of the exosome complex, an exoribonuclease complex of which the activity is essential for cell survival.

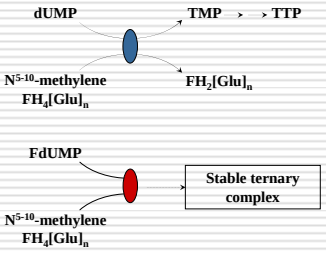
5-Fluorouracil

Activation pathways for 5-FU

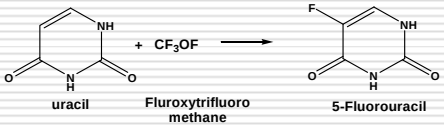


5-Fluorouracil

Thymidylate synthase inhibition by 5-FU



Synthesis of 5-Fluorouracil

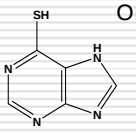


uracil Fluroxytrifluoro methane 5-Fluorouracil

6-Mercapto Purine

- Chemical Formula $C_5H_4N_4S$
- Trade Name Puninethol
- Dosage Forms

Tablet Oral



6-Mercaptopurine

6-Mercapto Purine

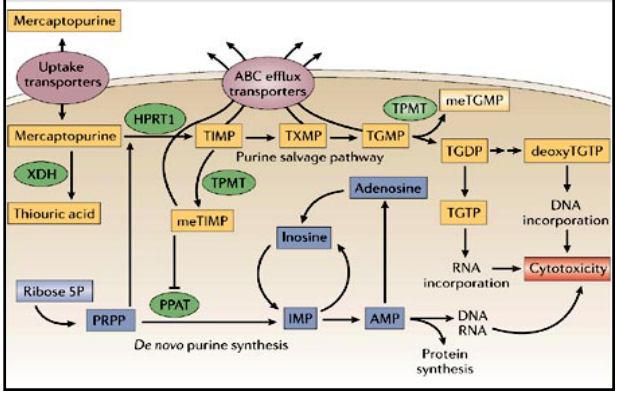
Pharmacology

- An antimetabolite antineoplastic agent with immunosuppressant properties. It interferes with nucleic acid synthesis by inhibiting purine metabolism and is used, usually in combination with other drugs, in the treatment of or in remission maintenance programs for leukemia.
- It is an analogue of the purine bases adenine and hypoxanthine.
- It is not known exactly which of any one or more of the biochemical effects of mercaptopurine and its metabolites are directly or predominantly responsible for cell death.

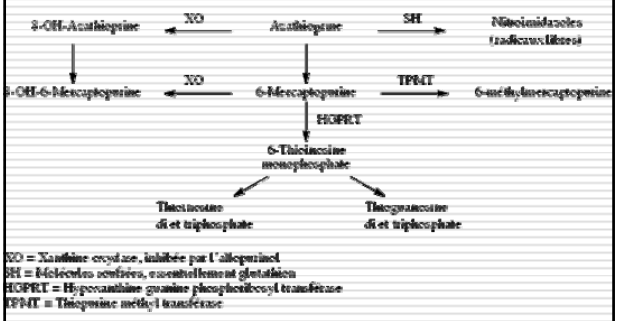
6-Mercapto Purine

- Mercaptopurine competes with hypoxanthine and guanine for the enzyme **hypoxanthine-guanine phosphoribosyltransferase** (HGPRTase) and is itself converted to **thioinosinic acid** (TIMP).
- This intracellular nucleotide inhibits several reactions involving inosinic acid (IMP), including the conversion of IMP to xanthylic acid (XMP) and the conversion of IMP to adenylic acid (AMP) via adenylosuccinate (SAMP).
- Some mercaptopurine is converted to nucleotide derivatives of 6-thioguanine (6-TG) by the sequential actions of inosinate (IMP) dehydrogenase and xanthylate (XMP) aminase, converting TIMP to thioguanylic acid (TGMP).

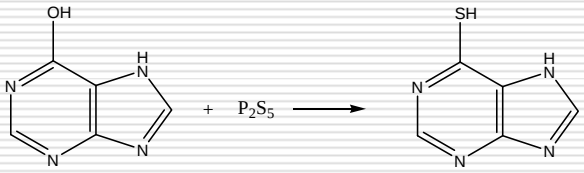
6-Mercapto Purine



6-Mercapto Purine



Synthesis of 6-Mercapto Purine



hypoxanthine 6-Mercaptopurine