Drug Discovery and Development

Molecular and Chemical Basis of

Cancer and Anti-Cancer Therapies

Christian Heinis

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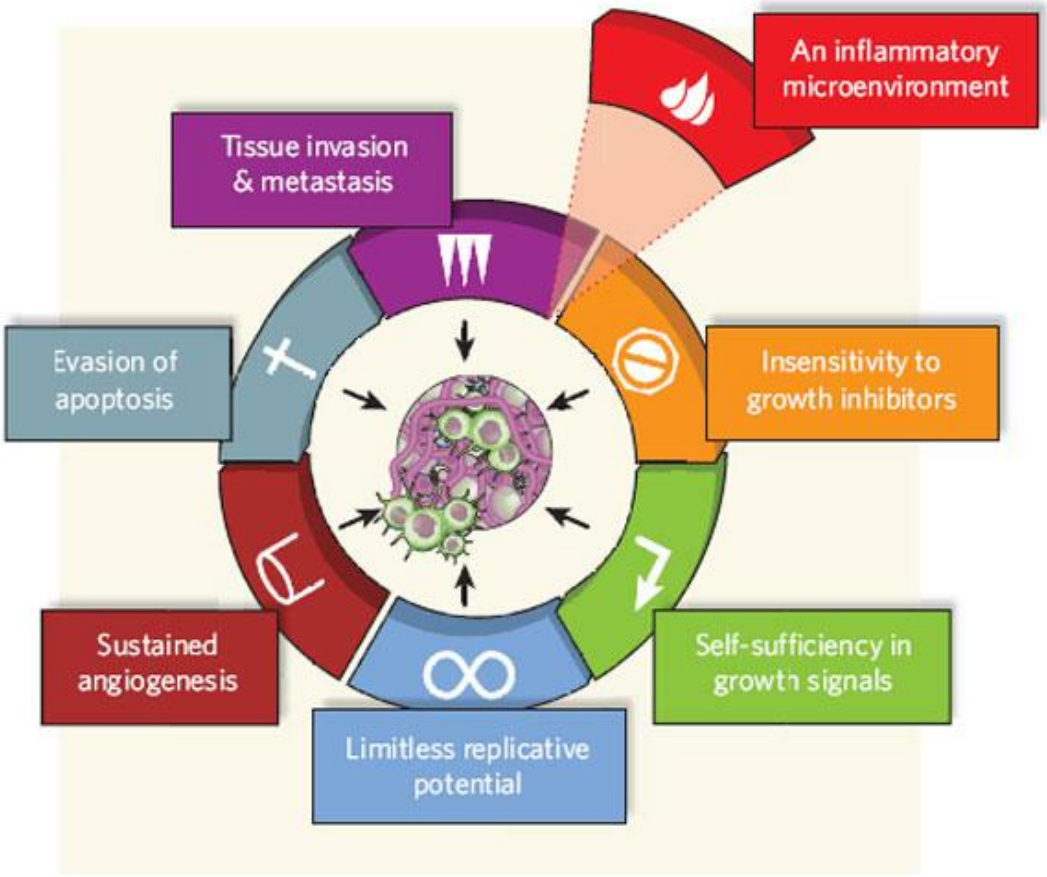
What is the difference between a healthy cell and a cancer cell?

The Hallmarks of Cancer



6 (or now 10) characteristics of cancer cells

The Hallmarks of Cancer

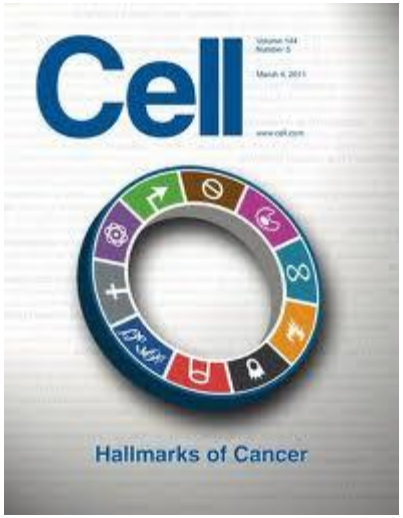
Article in the journal ‘Cell’ by Doug Hanahan and Robert Weinberg in 2000

Cited more than 10’000 times

Prof. Douglas Hanahan
ISREC Director

"Le Temps" of March 9, 2011

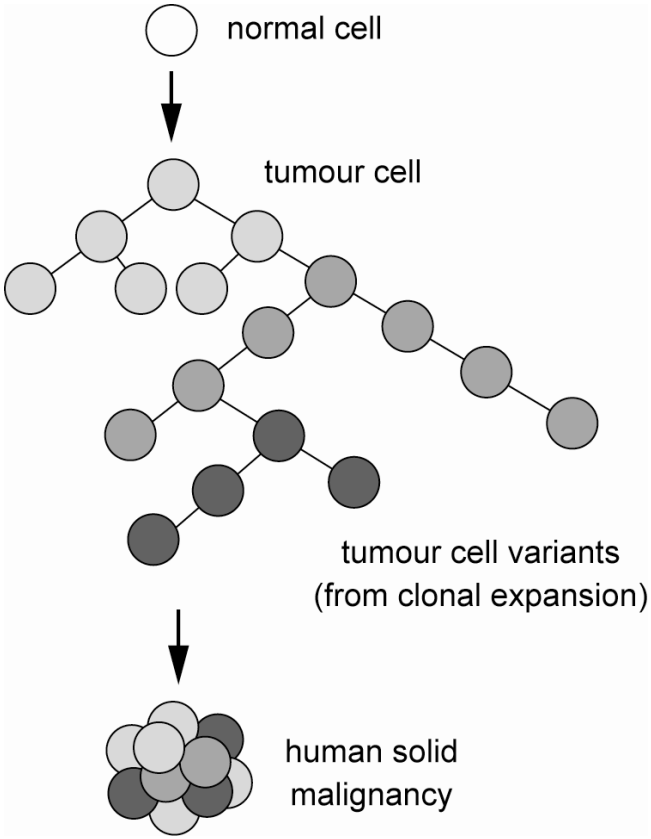
"Comment le cancer se développe..."
"The Hallmarks of cancer..."



How is a healthy cell converted into a cancer cell?

What are the molecular processes?

Clonal expansion



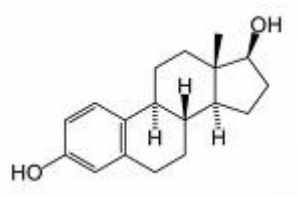
- Cells divide and accumulate more mutations
- Variants that evade the hoste defence grow faster

Which was the first anti-cancer drug that became available?

Early history of anti-cancer drugs

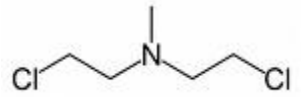
- First anti-cancer drug is a hormone (1941): estrogen

  (administration of estrogen produced regression of metastatic prostate cancer)

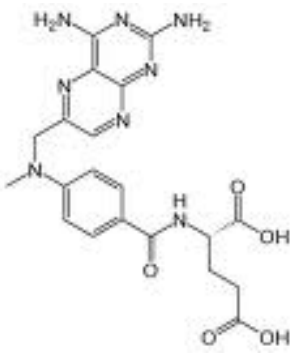


- A few years later: first alkylating agents

  (mechlorethamine was effective against Hodgkin's lymphoma)

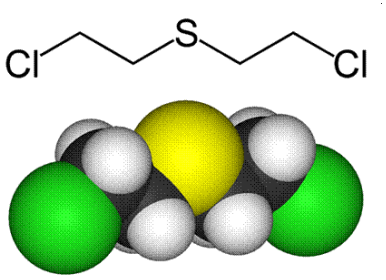


- 1950ies: antimetabolites (e.g. methotrexate, 5-fluorouracil), additional alkylating agents, natural products



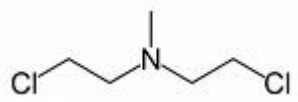
- 1960ies: progress continues in all areas

Alkylating agents

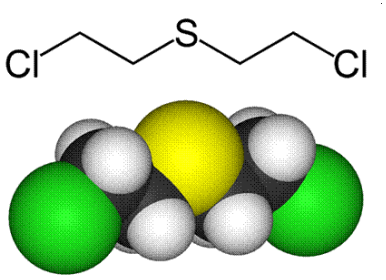


- Sulphur mustard was first used as chemical weapon (WWI)
- Sulphur mustard was active against animal tumours

  (but it was too toxic)
- 1946: Mechlorethamine was found active against Hodgkin's disease (lymph cancer)



Alkylating agents



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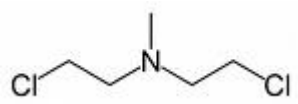
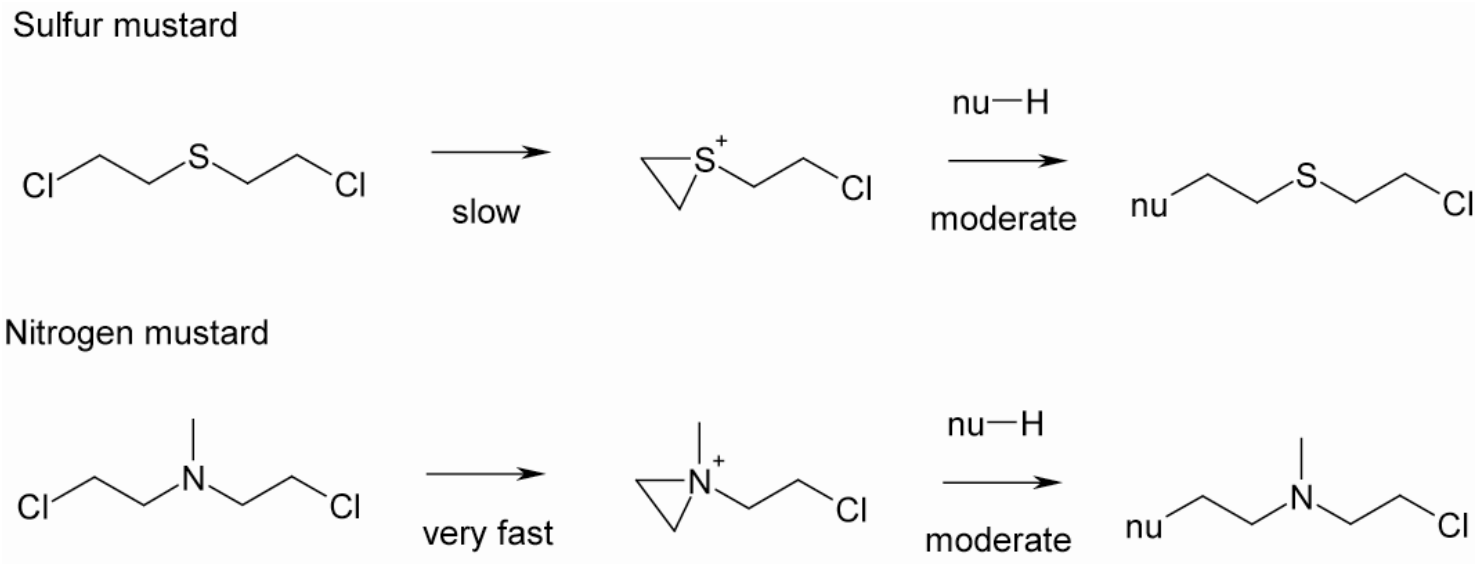
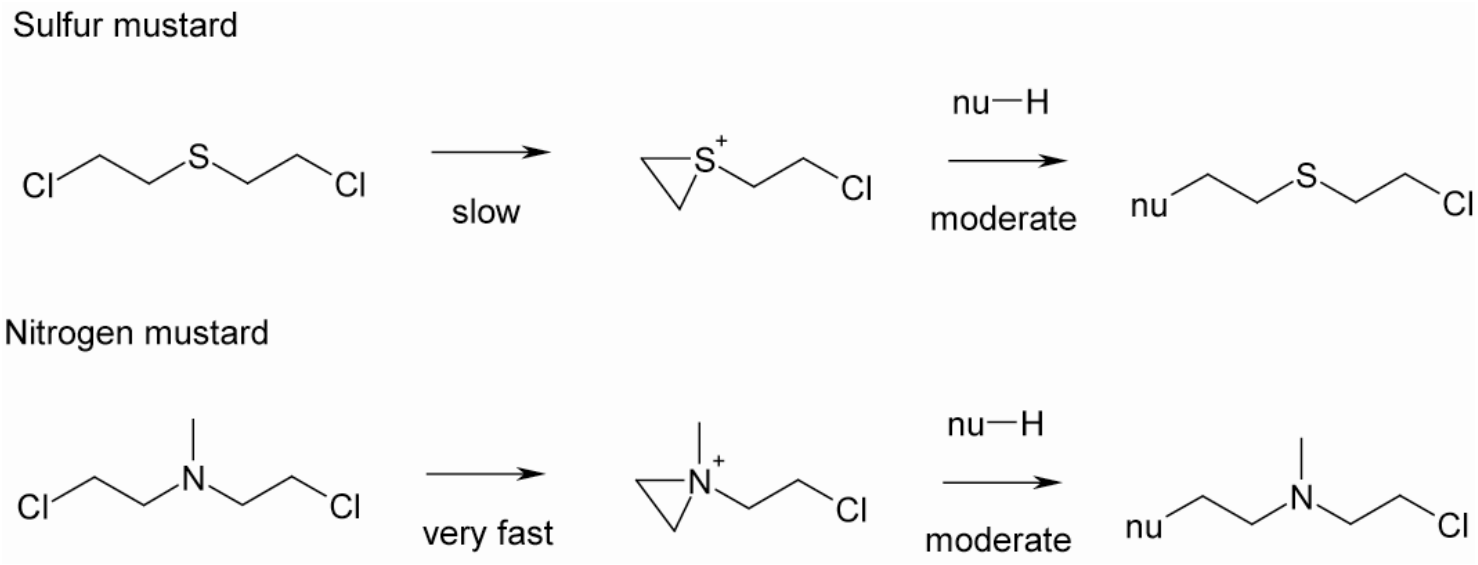


What is the mechanism of action of this drug?

Alkylating agents



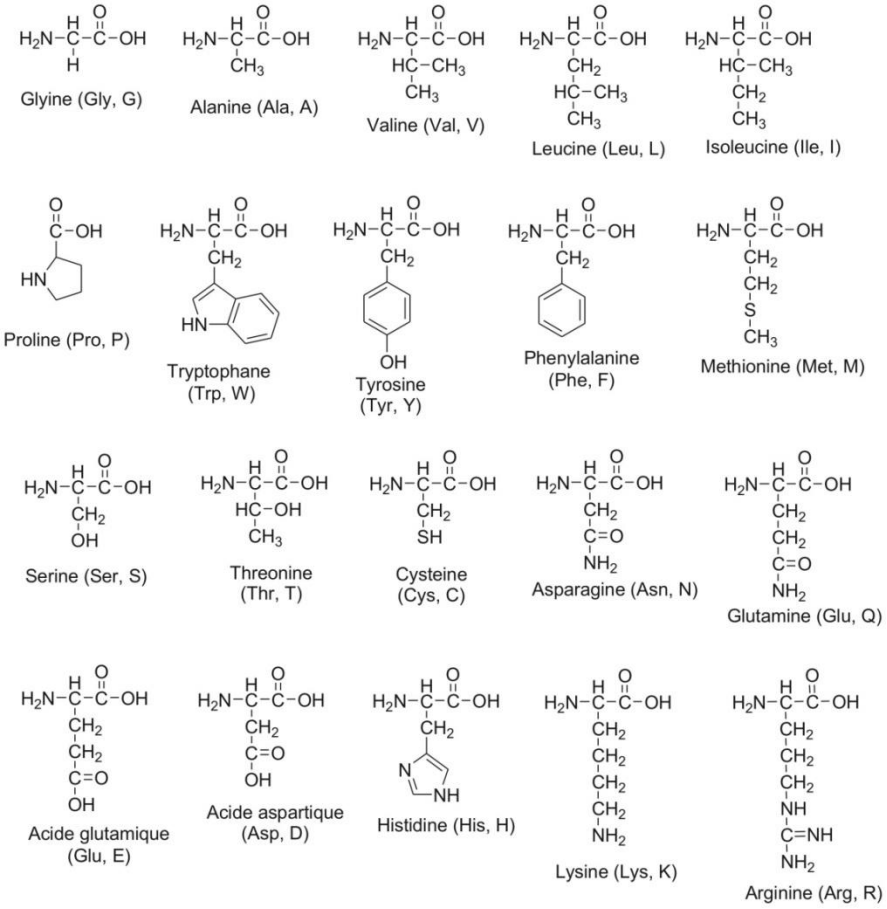
Alkylating agents



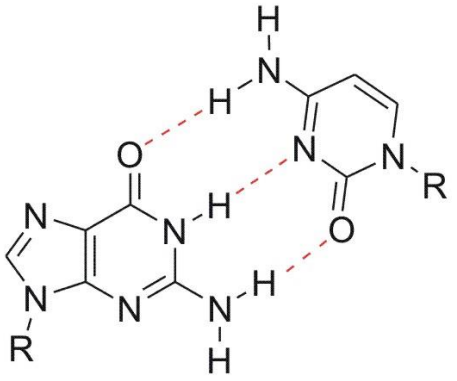
Which molecules are good nucleophiles in a cell?

Alkylating agents

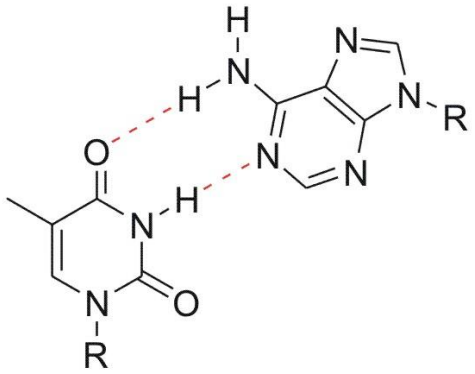
Protein / amino acids?



DNA / nucleotides?



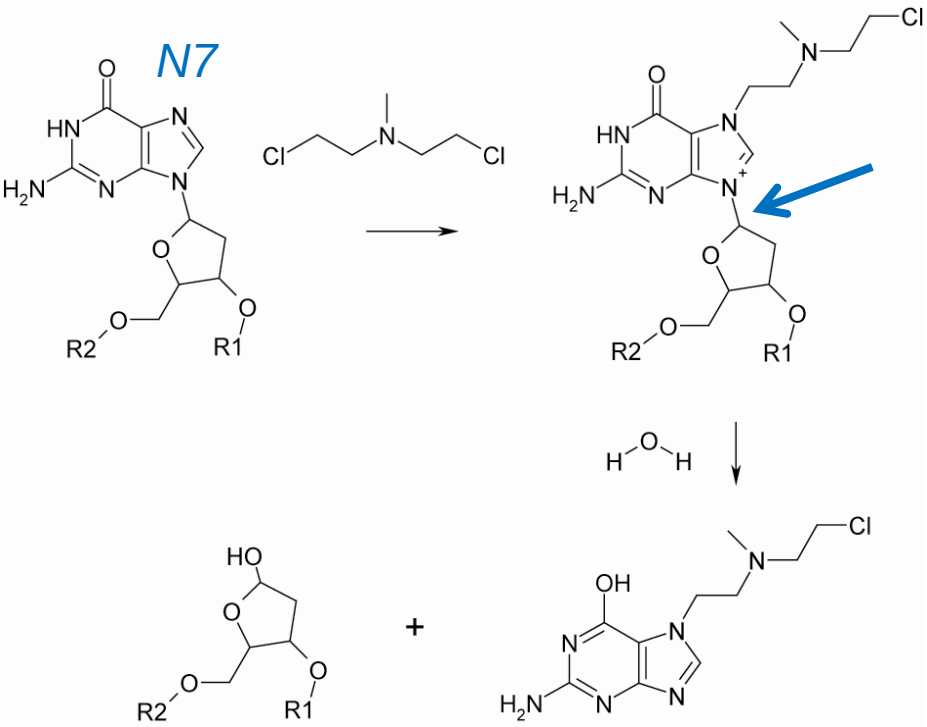
G-C



T-A

Alkylating agents: Mechanism of action

Guanine



Alkylating agents are thought to react mostly with DNA

Alkylating agents: Mechanism of action

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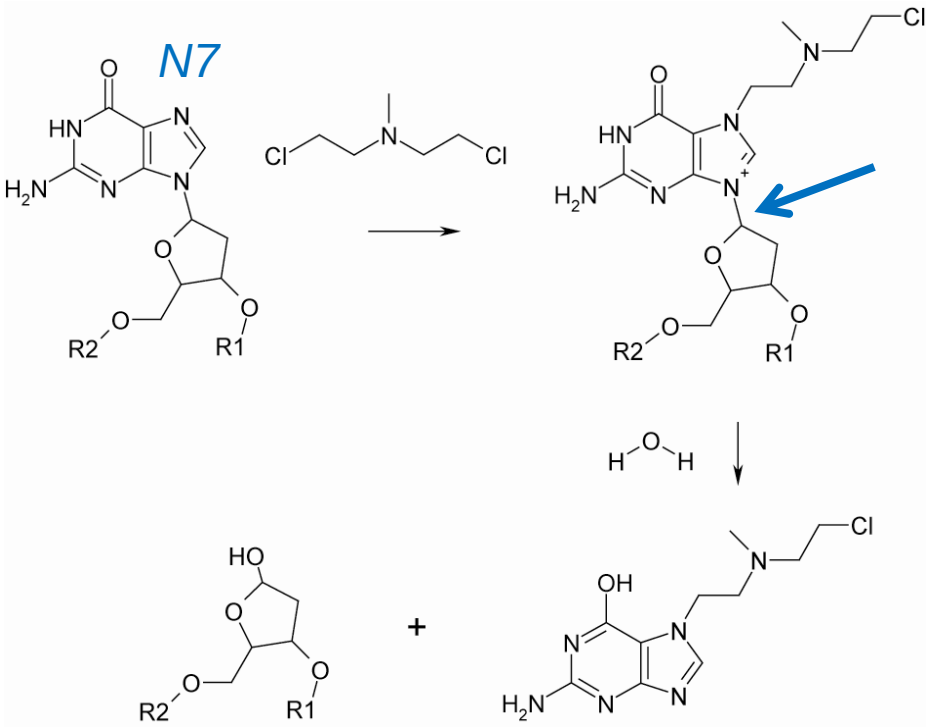

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Mechanisms:

- Mutagenesis
- Strand break
- Cross-linking

→ Faulse replication

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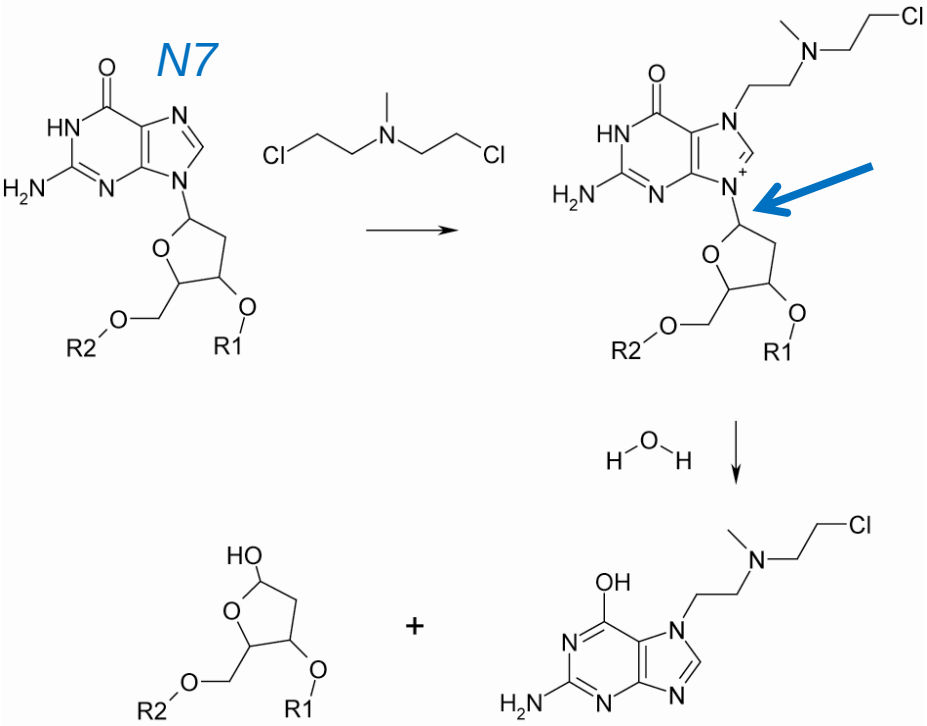

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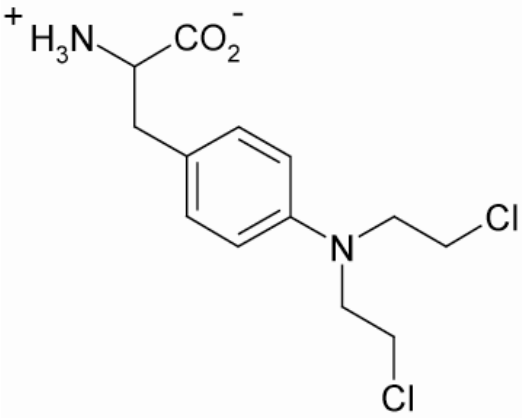
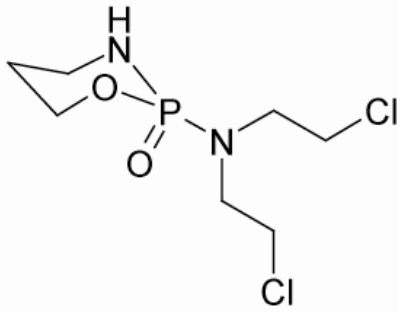
Why are cancer cells more affected by an alkylating agent than healthy cells?

Alkylating agents: Examples



melphalan



cyclophosphamide

Antimetabolites

- Compounds that prevent the biosynthesis of metabolites.

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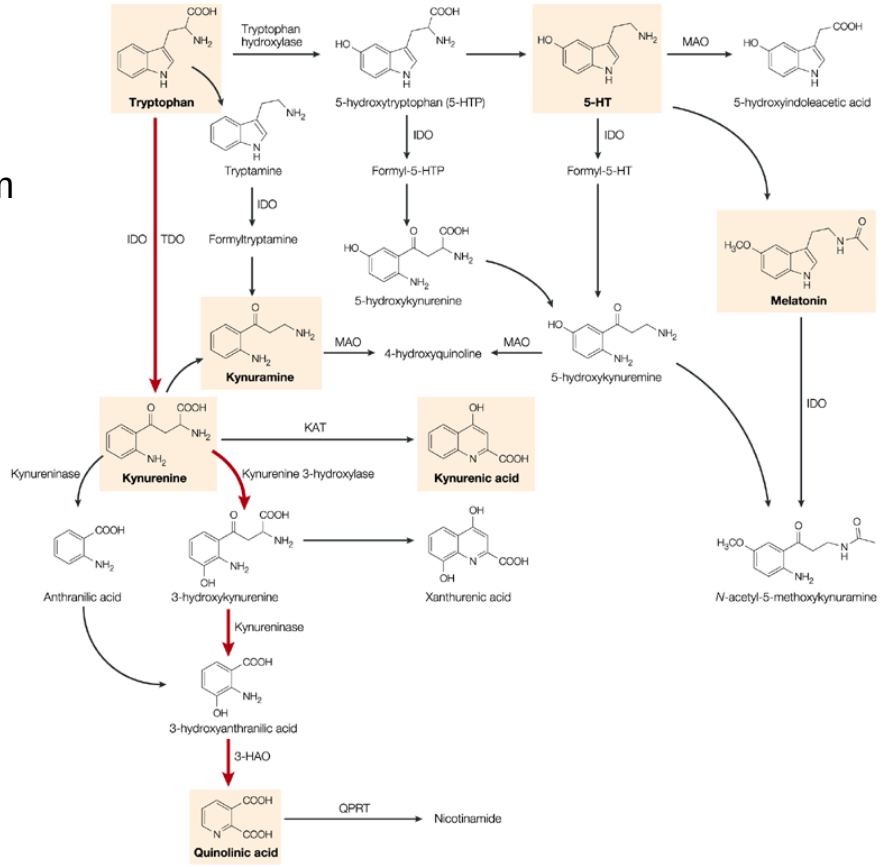
Example:

tryptophan metabolism



Antimetabolites

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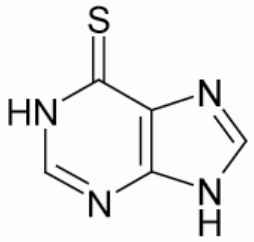

6-mercaptopurine

Antimetabolites

- Compounds that prevent the biosynthesis of metabolites.

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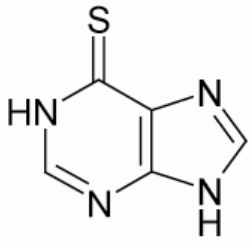
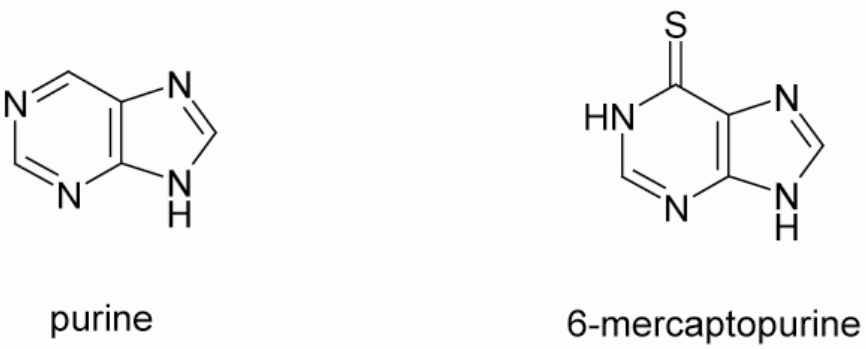


6-mercaptopurine

Which metabolite is similar to this antimetabolite ?

6-mercaptopurine

Example 1:

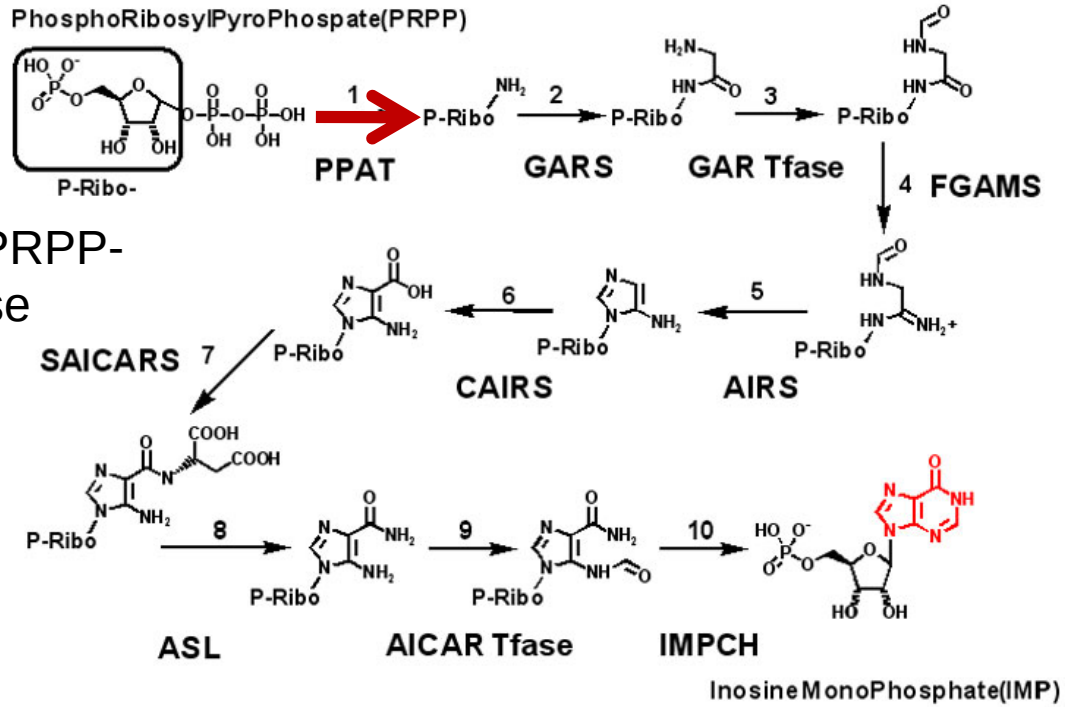


Which pathway does 6-mercaptopurine inhibit?

De novo purine synthesis

Phosphoribosyl pyrophosphate

Phosphoribosyl-amine

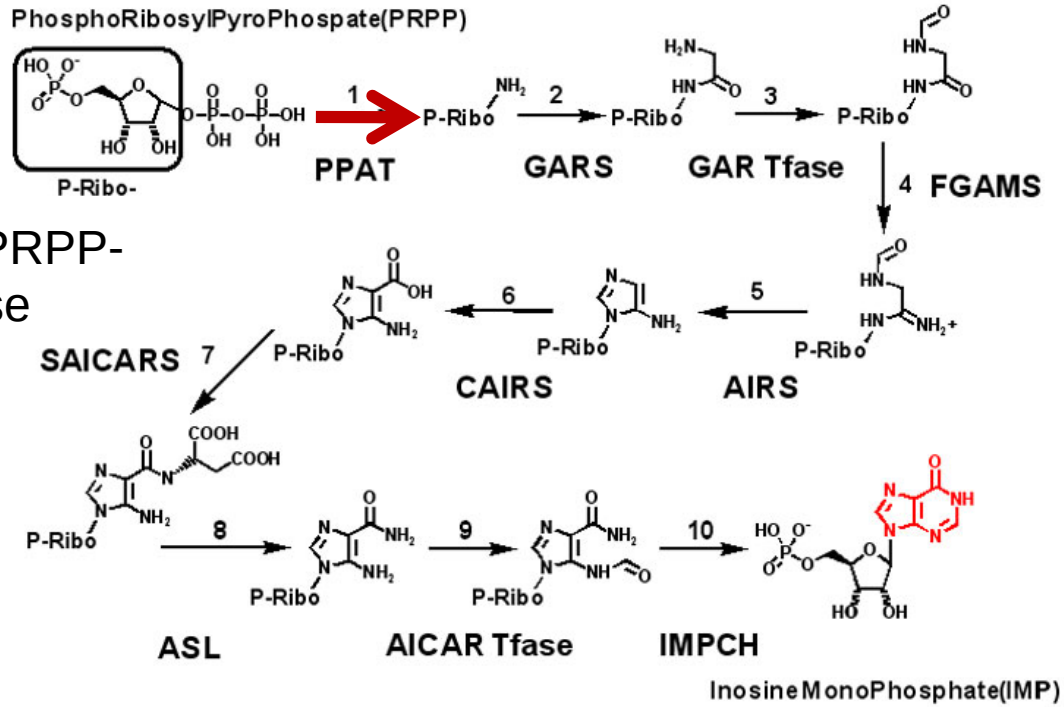


PPAT = Glutamine-PRPP-aminotransferase

De novo purine synthesis

Phosphoribosyl pyrophosphate

Phosphoribosyl-amine

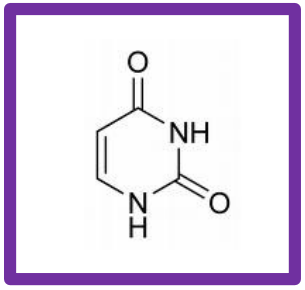


PPAT = Glutamine-PRPP-aminotransferase

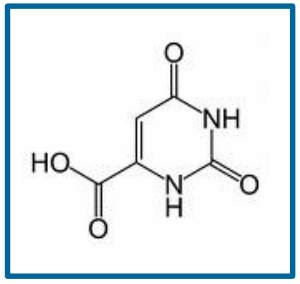
Why are cancer cells more affected by 6-mercaptopurine than healthy cells?

Example 2: **Rational design of an antimetabolite of uracil (1957)**

Observation: in certain tumours uracil was used more than orotic acid for nucleic acid biosynthesis



uracil



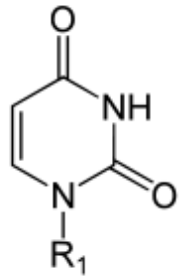
orotic acid

thymidine for

DNA synthesis

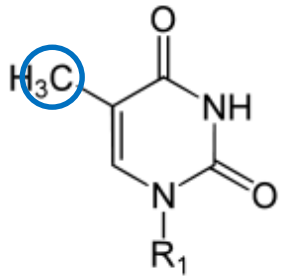
Convertion of uridylate to thymidylate

2’-deoxyuridylic acid



R_1 = 2-deoxyribose momophosphate

1a

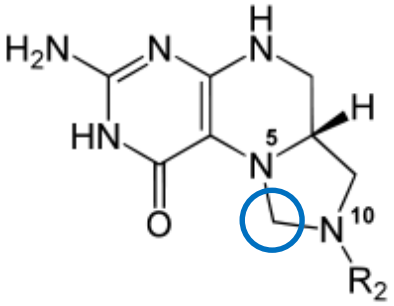


1b

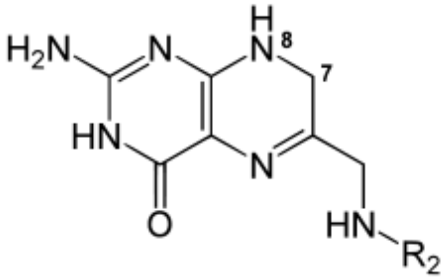
2’-deoxythymidylic acid

A

A = thymidylate synthase



2

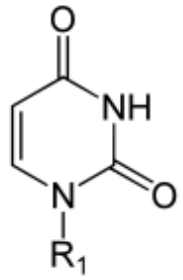


3

cofactor: 5,10-methylene-tetrahydrofolate

Convertion of uridylate to thymidylate

2’-deoxyuridylic acid



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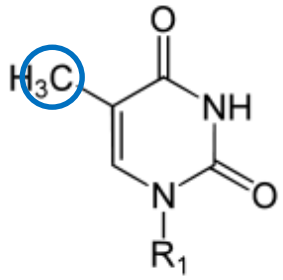

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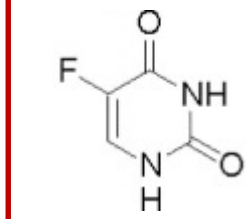
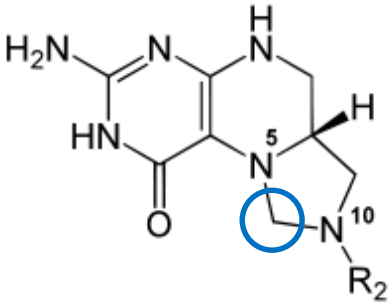


5-fluorouracil

A

A = thymidylate synthase



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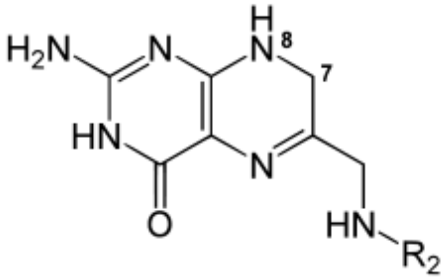
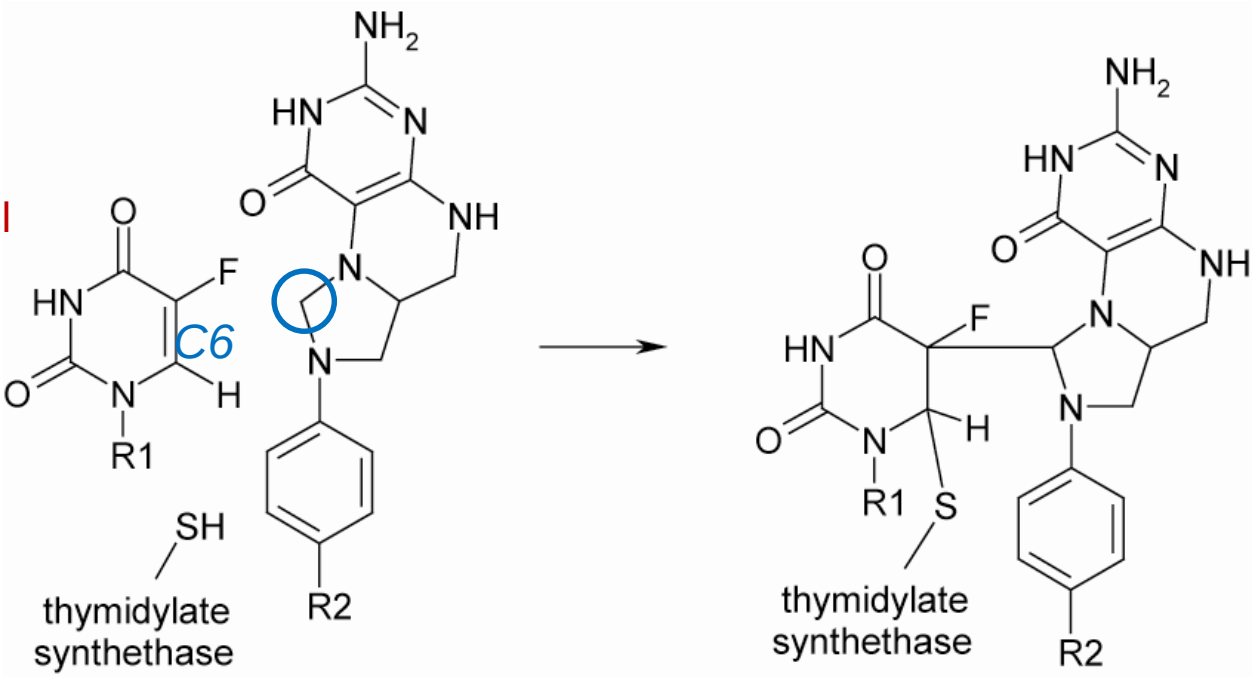


3

cofactor: 5,10-methylene-tetrahydrofolate

A different mechanism was found

5-fluorouracil

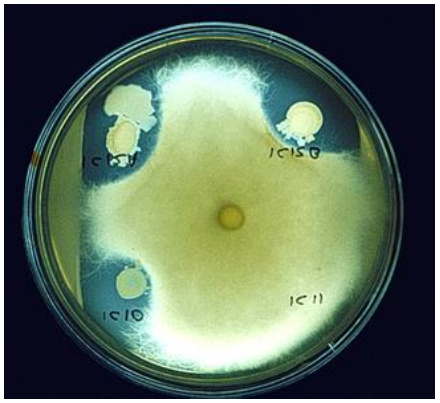


- The fluoro group prevents the full transfer of the methylene group
- Enzyme, cofactor and inhibitor form a covalent complex

  → thymidylate synthase is irreversibly inhibited

Antibiotics

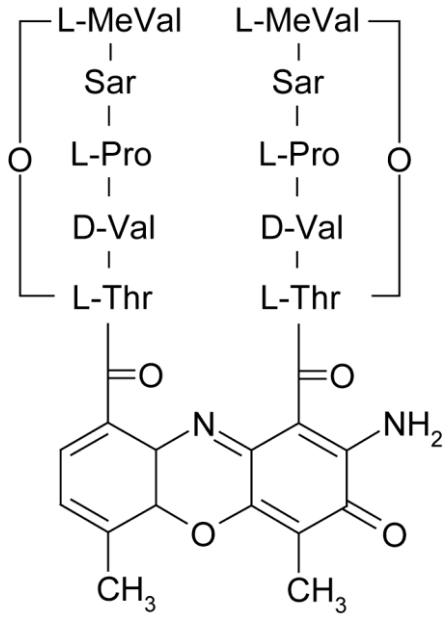
- Original definition of '*antibiotic*' = substance produced by a micro-organism that inhibits the growth of other micro-organisms



- Many antibiotics were too toxic to be used as anti-infectious agents
- Later, some antibiotics were found to be useful as anti-cancer drugs

Antibiotics

Example 1: **actinomycin D**



2 x pentapeptide lactone

3-phenoxazine-1,9-dicarboxylic acid

Antibiotics

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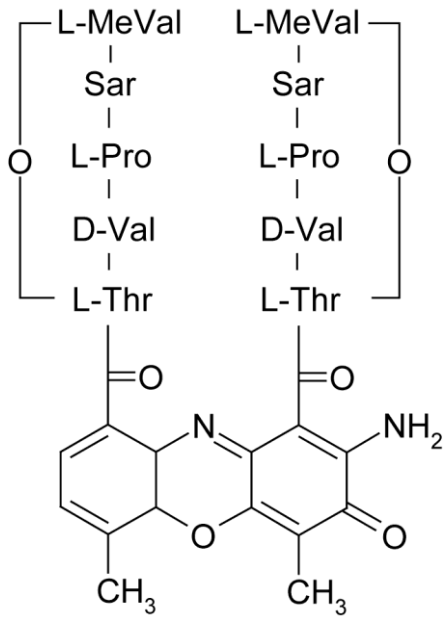
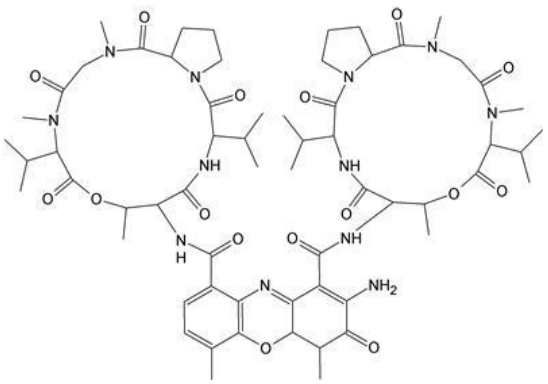


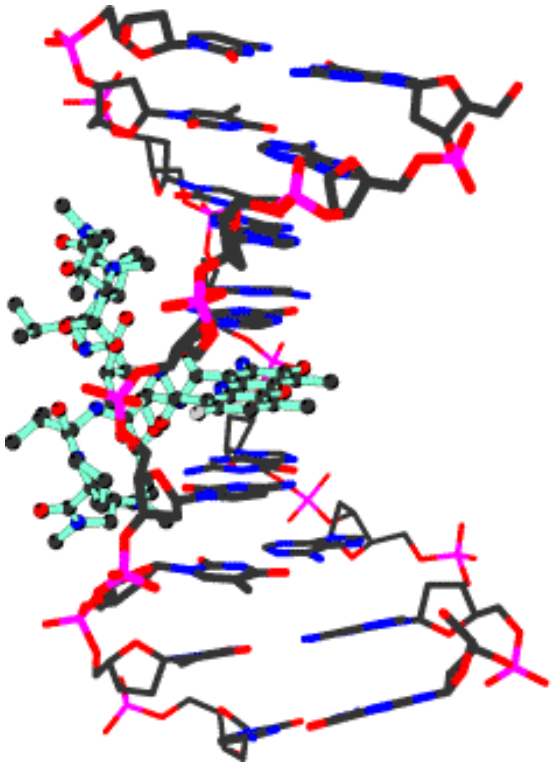
2 x pentapeptide lactone

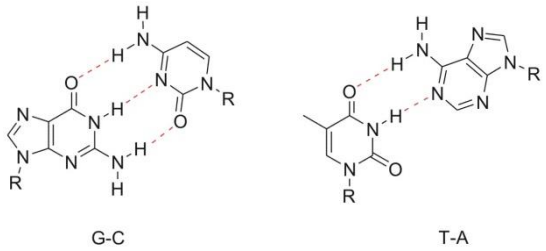
3-phenoxazine-1,9-dicarboxylic acid

What is the mechanism of action of actinomycin D?

Actinomycin D



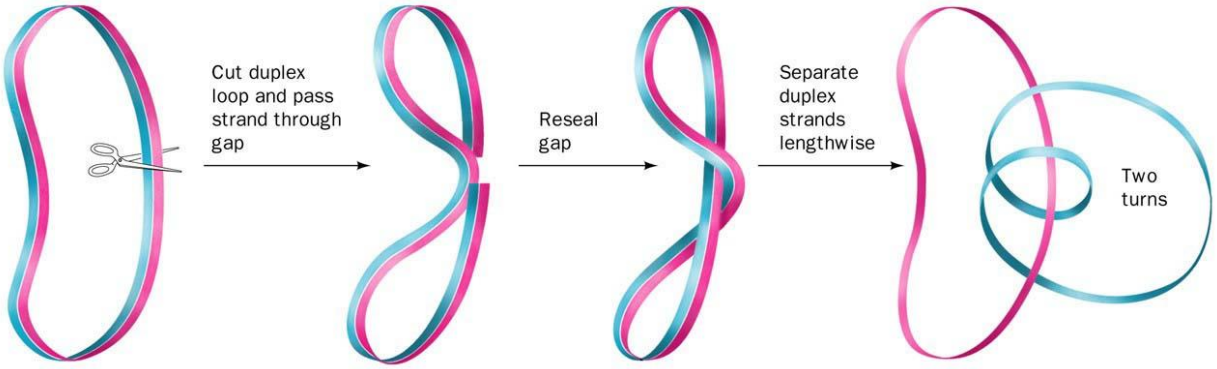


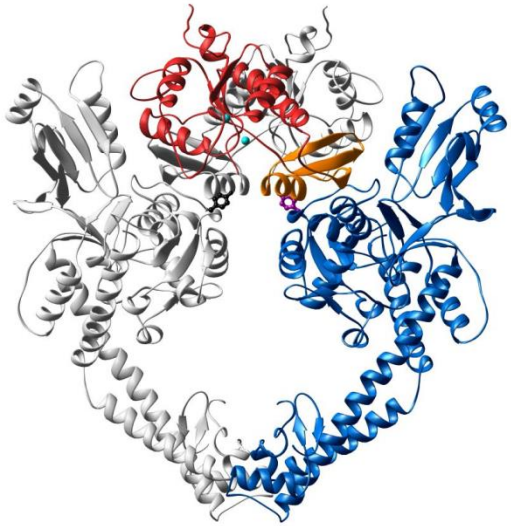


- Phenoxazine group intercalates into the double helix
- Double helix needs to unwind
- Pentapeptide-lactone groups bind into the minor grooves of the helix

  Why is the binding to the DNA toxic for the cell?

Actionmycin D inhibits topoisomerase II

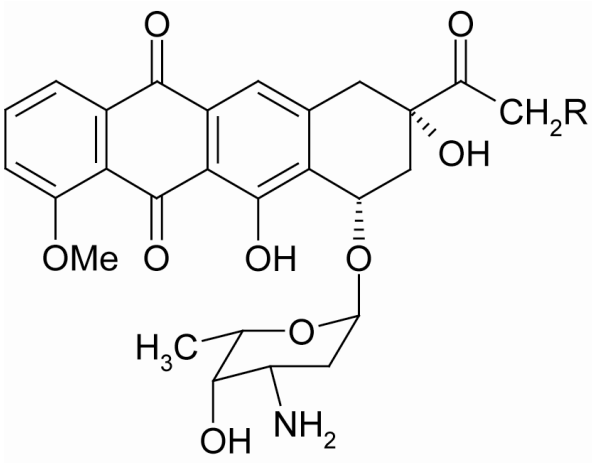




topoisomerase II

Antibiotics

Example 2: **anthracyclines**



daunorubicin: R = H

doxorubicin: R = OH

Antibiotics

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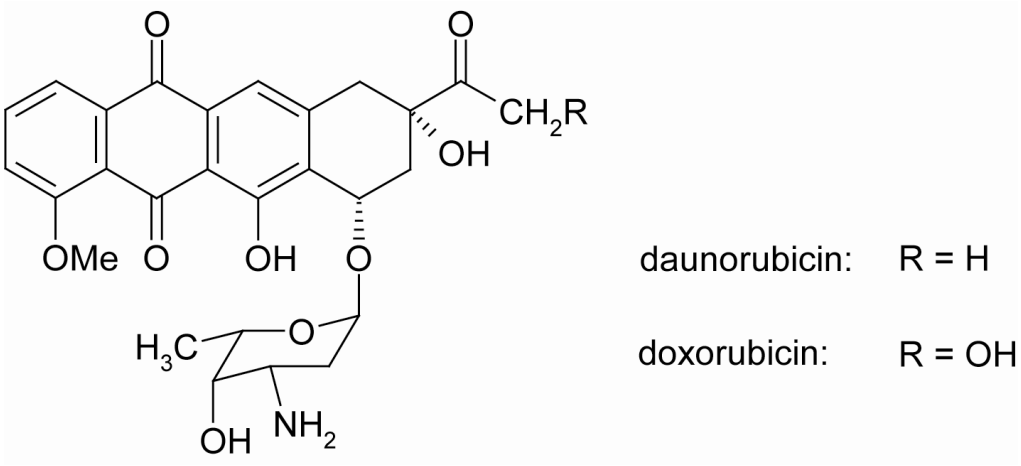
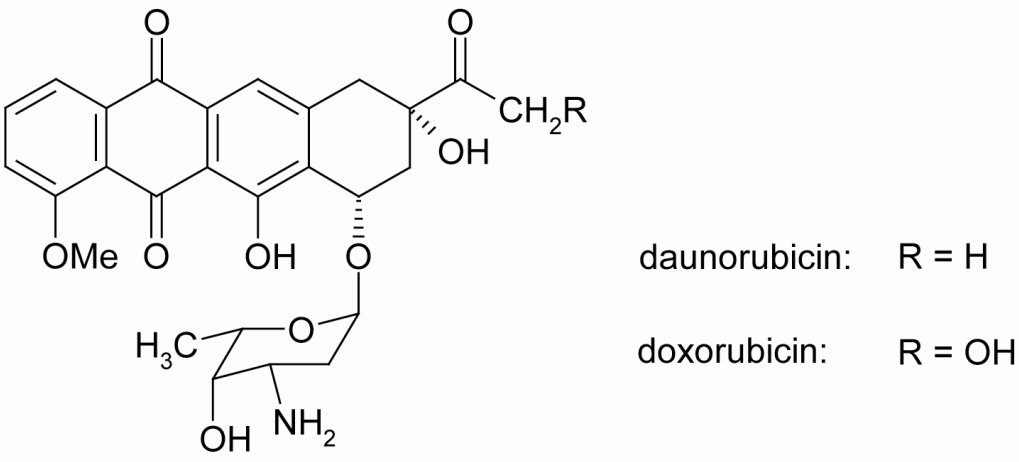


What is the mechanism of action of daunorubicin / doxorubicin?

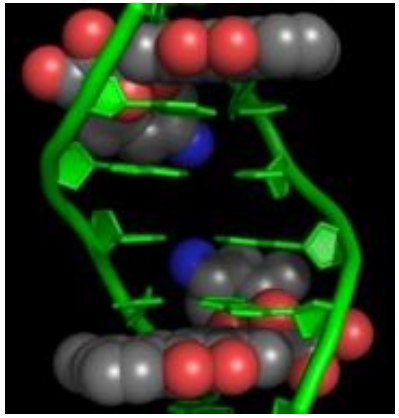
Antibiotics

Example 2: **anthracyclines**



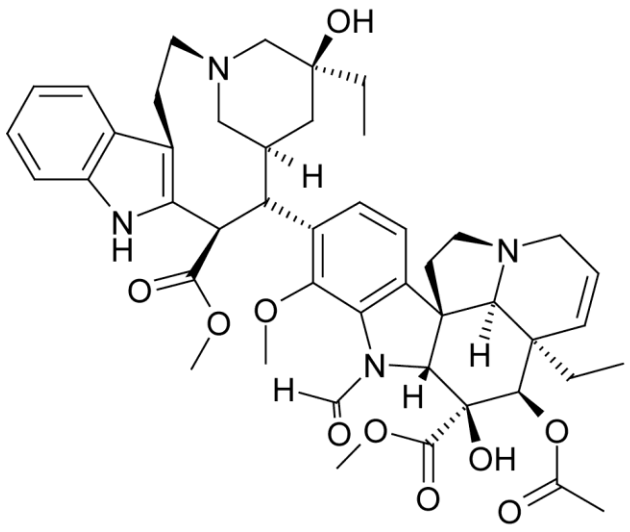
daunorubicin: R = H

doxorubicin: R = OH



Natural products

Example 1: **vinca alkaloids**

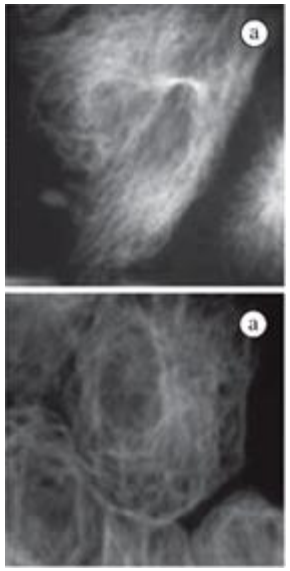


vincristine

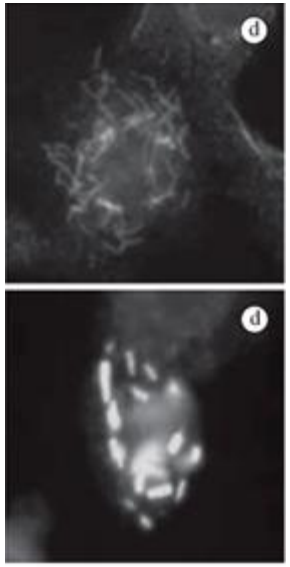
Catharanthus Roseus

Effect of vincristine

Cells are stained with an anti-tubulin antibody

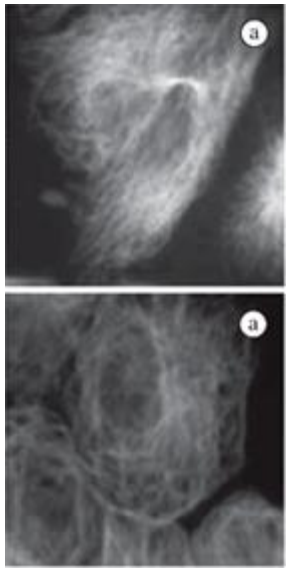


addition of
vincristine

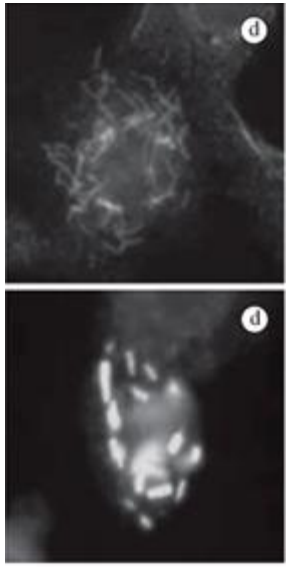


Effect of vincristine

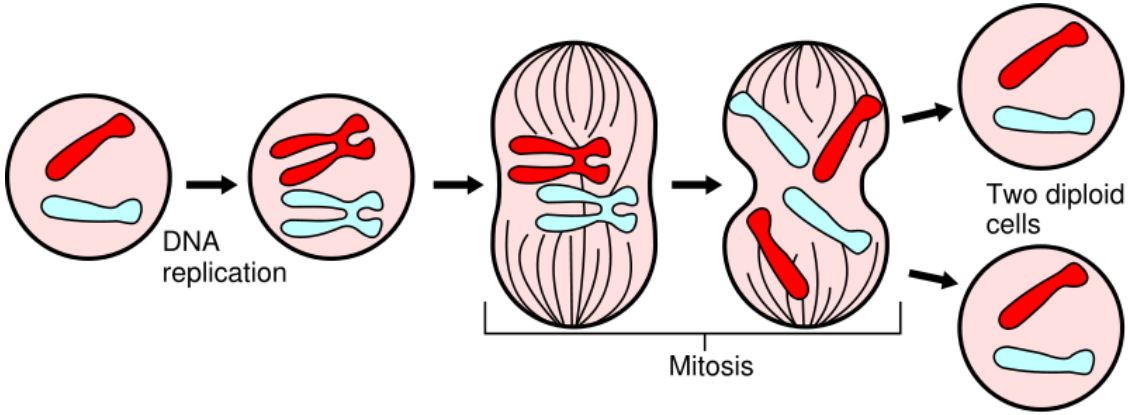
Cells are stained with an anti-tubulin antibody



addition of
vincristine



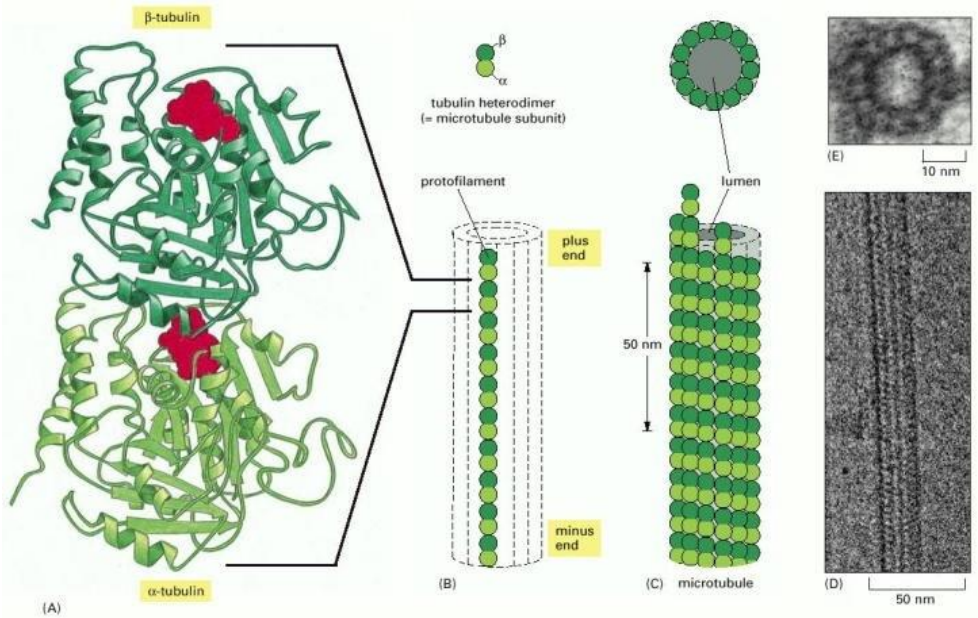
Mitotic arrest in cell cycle



Vincristine causes mitotic arrest by dissociating the

cytoskeleton and the **mitotic spindle**

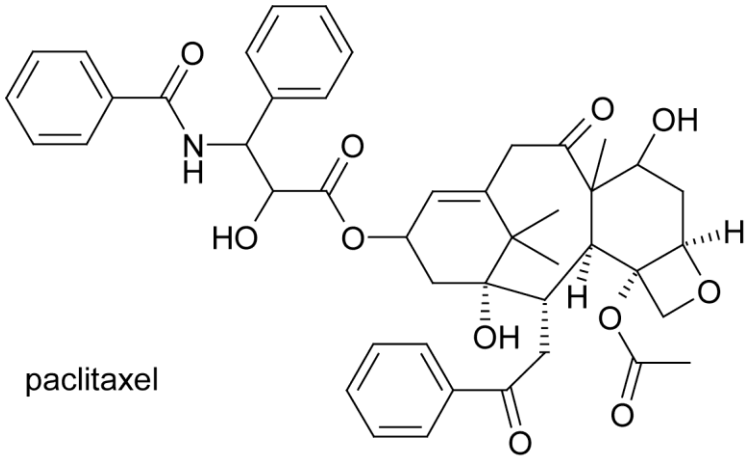
Vincristine binds to tubulin



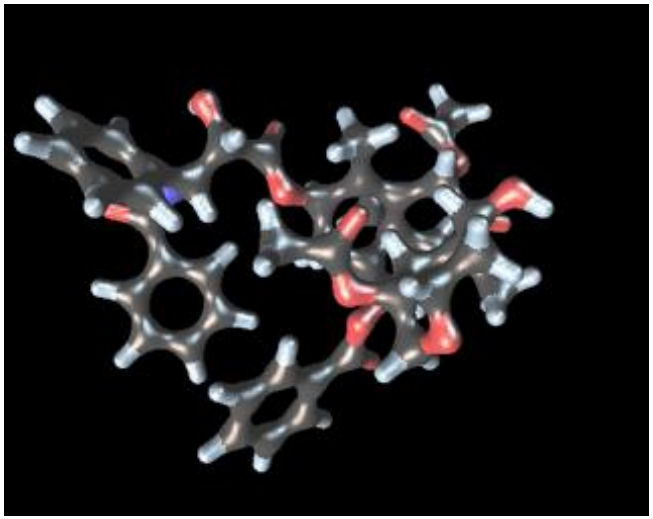
Vincristine promotes the dissociation of microtubuli

Natural products

Example 2: **paclitaxel (Taxol)**

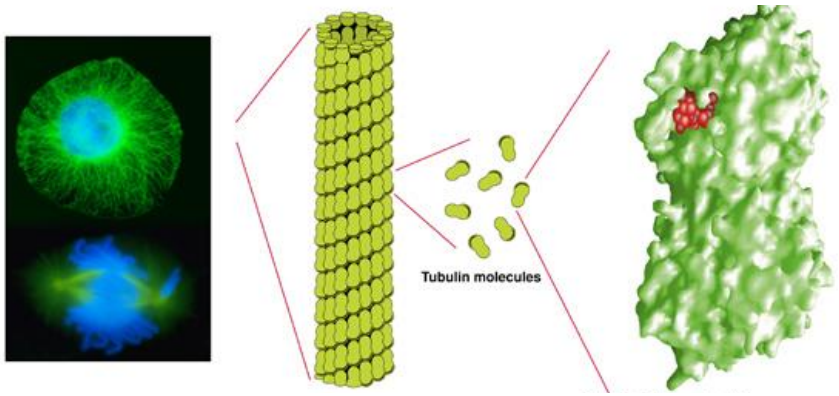


paclitaxel



Was world's leading small molecule anti-cancer agent in terms of sales.

Inhibition of depolymerization



Taxol promotes the assembly of microtubulus and inhibits their depolymerization

→ Contrary mechanism of vinca alkaloids

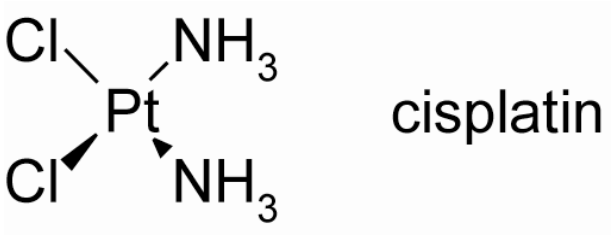
Paclitaxel was isolated from a yew tree (1971)

Bark of the Pacific yew tree *Taxus brevifolia*

- *Taxus brevifolia* is a slow growing tree and contains only small amounts of the drug → small yields
- Bristol-Myers Squibb (BMS) developed a semi-synthetic route:
  - Isolation of 10-deacetylbaccatin III from needles of *Taxus baccata* (a European yew tree)
  - Chemical transformation into paclitaxel

Platinum complexes

Example: **cisplatin**

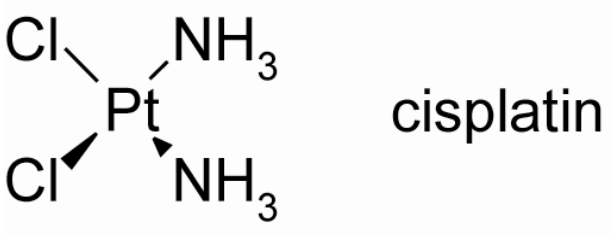


cisplatin

- Toxicity was discovered by accident when the effect of an electrical field on bacterial cells was tested:
  Bacterial cells formed long filaments
- Not the electrical field caused the toxicity but a complex of platinum and ammonium and chloride ions

Platinum complexes

Example: **cisplatin**



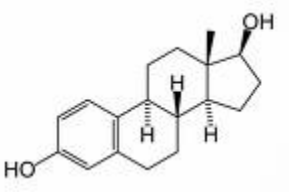
cisplatin

- Toxicity was discovered by accident when the effect of an electrical field on bacterial cells was tested:
  Bacterial cells formed long filaments
- Not the electrical field caused the toxicity but a complex of platinum and ammonium and chloride ions

What is the mechanism of action of cisplatin?

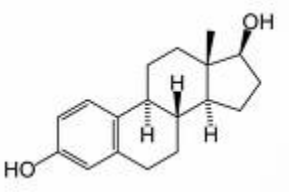
Hormones

Example: **estrogen**



Hormones

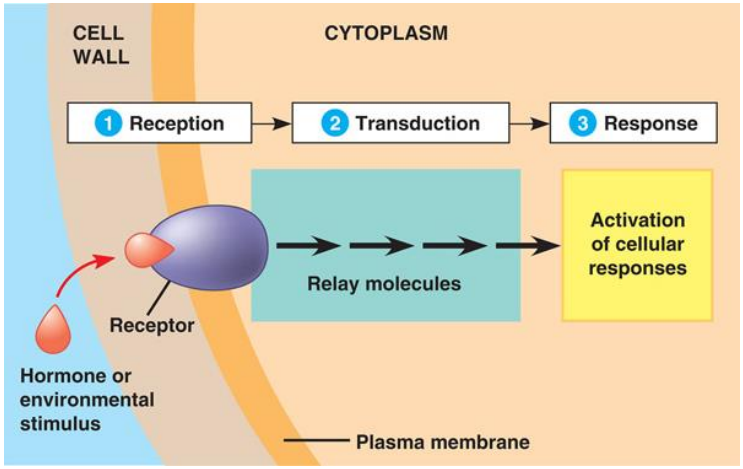
Example: **estrogen**

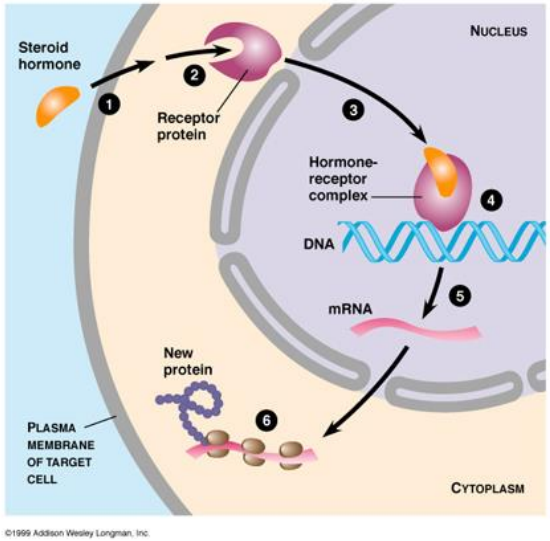


Why can hormones be used as anti-cancer drugs?

Hormones

- Normal and well-differentiated neoplastic cells have a number of hormone receptors

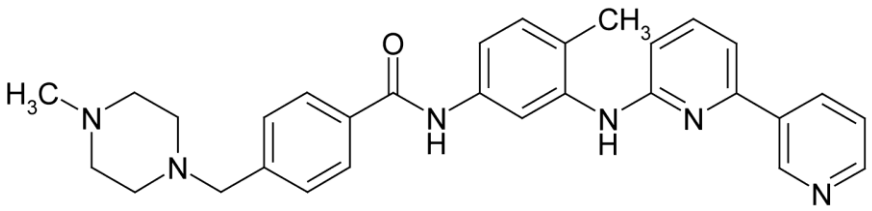




- Growth of cells can be regulated by hormones
- Many tumours are not differentiated and their growth can not be controlled by hormones

Signal transduction inhibitors

Example: **glivec**



imatinib (Gleevec)

Case study:

GLIVEC (STI571, IMATINIB), A RATIONALLY DEVELOPED, TARGETED ANTICANCER DRUG

Renaud Capdeville, Elisabeth Buchdunger, Juerg Zimmermann and Alex Matter

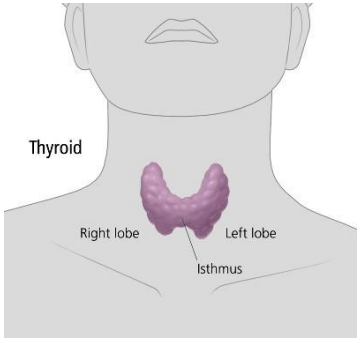
In the early 1980s, it became apparent that the work of pioneers such as Robert Weinberg, Mariano Barbacid and many others in identifying cancer-causing genes in humans was opening the door to a new era in anticancer research. Motivated by this, and by dissatisfaction with the limited efficacy and tolerability of available anticancer modalities, a drug discovery programme was initiated with the aim of rationally developing targeted anticancer therapies. Here, we describe how this programme led to the discovery and continuing development of Glivec (Gleevec in the United States), the first selective tyrosine-kinase inhibitor to be approved for the treatment of a cancer.

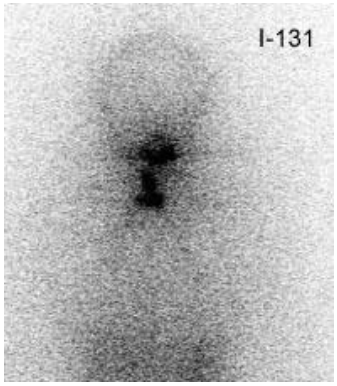
Until the early 1980s, drug discovery programmes for cancer were focused almost exclusively on DNA synthesis and cell division, and resulted in agents such as

The molecular consequence of this inter-chromosomal exchange is the creation of the *BCR–ABL* gene, which encodes a protein with elevated tyrosine-kinase activity.

Radiotherapeutic agents

- Produce beta particles (electrons)
- Travel 3 mm and destroy cell components
- Distribute to thyroide or bone → are used to treat cancer of these organs
- Examples: sodium phosphate (P32), sodium iodide (I131)





Monoclonal antibodies

- Proteins that have a Y-shape
- Bind to foreign objects (bacteria, viruses etc)
- Act through inactivation or signalling
- Produced by B cells
- In blood or bodily fluids
- **Can bind with high affinity and specificity to (protein) targets**

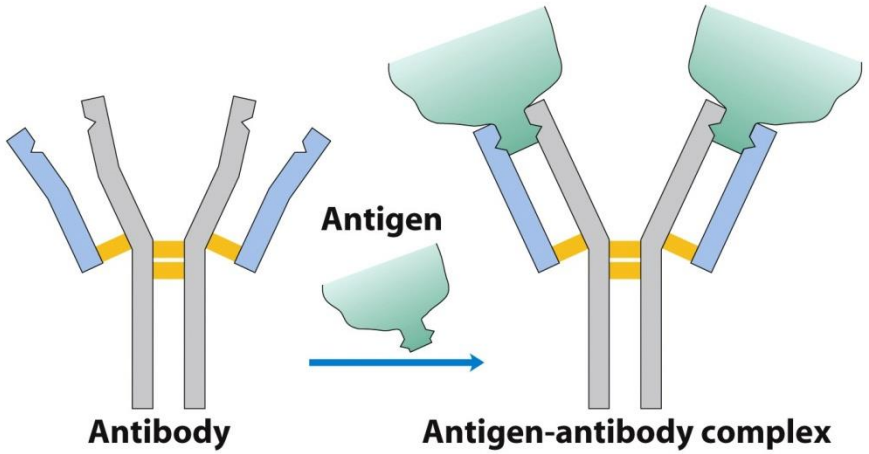
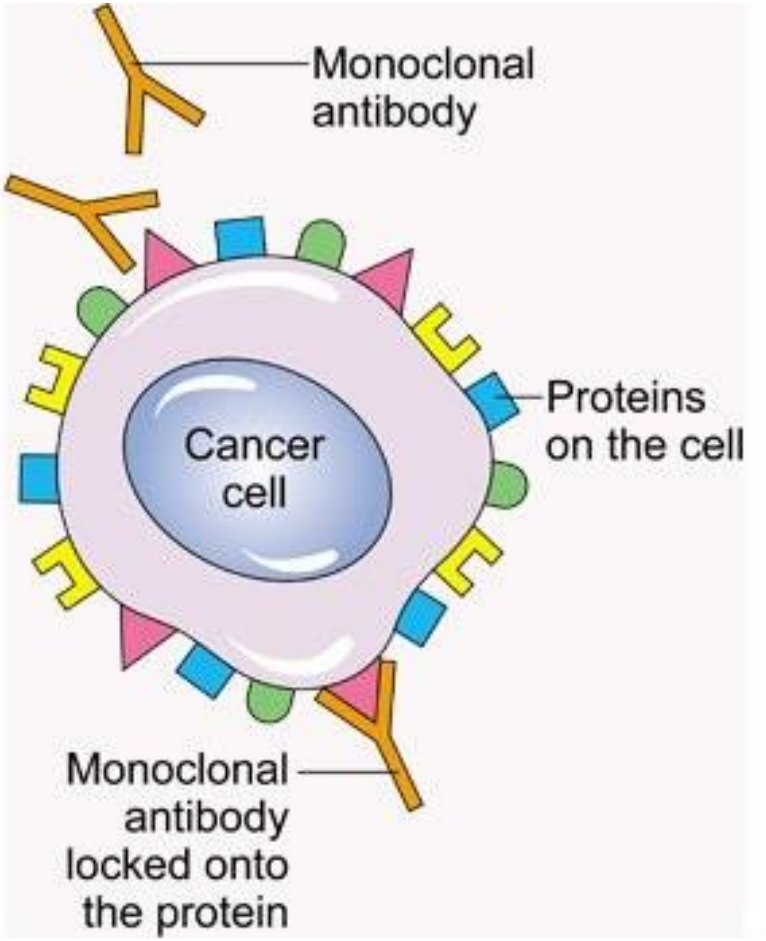


Figure 5-22
Lehninger Principles of Biochemistry, Fifth Edition

Antibodies with tailored binding specificities can be engineered



Monoclonal antibodies

Generic name	Description	Target	Approval date
Rituximab	Chimeric IgG1	CD20	1997
Trastuzumab	Humanized IgG4	HER2	1998
Gemtuzumab ozogamicin	Humanized IgG1	CD33 (immunotoxin)	2000
Alemtuzumab	Humanized IgG1	CD52	2001
Ibritumomab tiuxetan	Murine IgG1	CD20 (radiolabeled)	2002
^{131}I-Tositumomab	Murine IgG2	CD20 (radiolabeled)	2003
Cetuximab	Chimeric IgG1	EGFR	2004
Bevacizumab	Humanized IgG1	VEGF	2004
Panitumumab	Human IgG2	EGFR	2006
Ofatumumab	Human IgG1	CD20	2009
Ipilimumab	Human IgG1	CTLA-4	2011
Denosumab	Human IgG2	RANK ligand	2010
Brentuximab vedotin	Chimeric IgG1	CD30	2011
Pertuzumab	Human IgG1	HER2	2012
Obintuzumab	Humanized and glycoengineered	CD20	2013
Trastuzumab emtansine	Humanized IgG4	HER2 (mertansine)	2013
Ramucirumab	Human IgG1	VEGFR2	2014

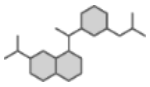
6 out of 10 top-selling drugs are mAbs

Year 2000

Drug	Sales (billion $)	Modality
Nexium	5.3	
Lipitor	5.3	
Plavix	4.7	
Advair	3.7	
Oxycontin	3.6	
Abilify	3.5	
Singulair	3.3	
Seroquel	3.2	
Crestor	2.9	
Cymbalta	2.6	

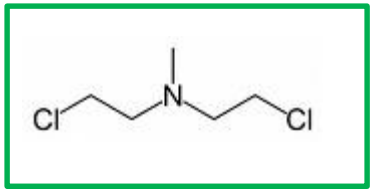
Year 2014

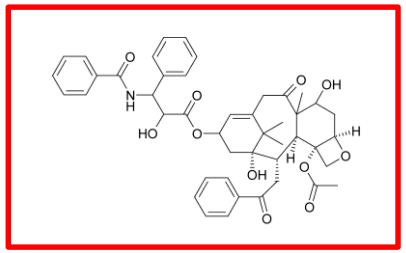
Drug	Sales (billion $)	Modality
Humira	12.5	
Solvadi	10.3	
Remicade	9.2	
Rituxan	8.7	
Enbrel	8.3	
Lantus	7.3	
Avastin	7.0	
Herceptin	6.8	
Advair	6.4	
Crestor	5.9	

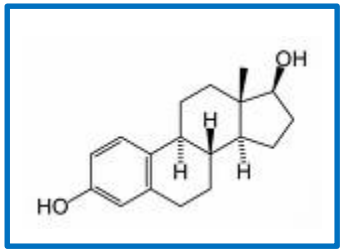
small molecule  antibody  peptide 

www.drugs.com/top200.html

Summary of anti-cancer drug classes







- Alkylating agents
- Antimetabolites
- Natural products
- Platinum complexes
- Hormones
- Signal transduction inhibitors
- Radiotherapeutic agents
- Monoclonal antibodies