Antibacterial and Antifungal Drugs

MODULE 06763
Semester 2

Department of Chemistry
University of Hull
ANTIBACTERIAL AND ANTIFUNGAL DRUGS
Course Synopsis

1. INTRODUCTION TO ANTIMICROBIAL AGENTS
   background and brief history
   reasons for studying antimicrobial chemotherapy
2. NON-SPECIFIC ANTIMICROBIAL AGENTS
   disinfectants, antiseptics and preservatives
3. ANTIBIOTICS AND ANTIBACTERIALS
   chemotherapy, course structure and the microbes
   3.i COMPETITIVE INHIBITORS (ANTIMETABOLITES)
   sulfonamides and miscellaneous antibacterials
   3.ii AGENTS ACTING AGAINST THE CELL MEMBRANE
   polypeptides
   3.iii AGENTS ACTING AGAINST NUCLEIC ACIDS
   quinolones and ansamycins
   3.iv AGENTS DISRUPTING PROTEIN BIOSYNTHESIS
   tetracyclines, macrolides, aminoglycosides, chloramphenicol, streptogramins
   3.v AGENTS ACTING AGAINST CELL-WALL BIOSYNTHESIS (PEPTIDOGLYCAN BIOSYNTHESIS)
   β-lactams (penams, cephehems, carbapenams, monobactams)
   glycopeptides
   mode of action of β-lactams and glycopeptides
4. BACTERIAL RESISTANCE
   4.i Modes of bacterial resistance
   4.ii β-lactamases and β-lactamase inhibitors and glycopeptide resistance
5. ANTIFUNGAL AGENTS
   5.i FUNGI AND FUNGAL INFECTIONS
   5.ii AGENTS ACTING AGAINST THE CELL NUCLEUS
   Griseofulvin and 5-fluorocytosine
   5.iii ERGOSTEROL
   Role in cell-wall structures, biosynthesis and key target enzymes
   5.iv AGENTS ACTING AGAINST THE FUNGAL CELL WALL
   Polyene antibiotics: Macrocyclic lactones
   5.v AGENTS AFFECTING ERGOSTEROL BIOSYNTHESIS
   thiocarbamates and allylamine derivatives
   morpholine derivatives
   imidazole and triazole derivatives

BOOKS AVAILABLE IN THE LIBRARY

   QP801 A63 F8. Good introductory coverage; especially useful in Part I
   RM409 R9 Greater depth and detail; of interest for 'reading around' the subject
   RM267 M2 Elementary overview; useful on chemical topics; deficient/unreliable on others
   RM409 D2 Wealth of up-to-date information, includes bacterial and clinical aspects
   QD 401 Z8 Dry as dust.

APPENDICES

Appendix A  Bacterial infections
Appendix B  Drugs to treat tuberculosis and leprosy
Appendix C  Fungal infections
Appendix D  Glossary
INTRODUCTION

Fungi and bacteria are everywhere: in the soil, on trees, in grass and in woodlands, the fur on our pets, our hair, they are on our skin and in our intestinal tract. For example **coccidiomycosis** is an infection endemic in certain arid/desert areas of the USA. About 40 million people are infected with **Histoplasma capsulatum** - in some areas up to 90% of people show a positive skin test to **Histoplasma**.

Fungi and bacteria can be **saprophytes** (living on dead so causing the decay of foods, fabrics and timber), but many others are **parasites**, some of which are human parasites. We mostly co-exist happily with these microbes, but they can proliferate on or in the outermost layers of skin causing irritation or growths (fungi), and in extreme cases serious infection.

The **first recorded account** of a human fungal infection was by **Hippocrates** (460-377 B.C.) - a case of oral candidiasis, which was known as 'thrush' from around the time of **Samuel Pepys**. In the same year a London bulletin 'Diseases and Casualties of the Week'. London, from the 12th of September to the 19th, 1665 reported the following causes of deaths, including the **first documented case of a fatality due to a fungal infection**:

| Consumption 129, Fever 332, Plague 6544, Thrush 6... |

**Robert Hooke**: described the first detailed examination of a fungus in **Micrographia** (1665), his famous treatise on the newly invented microscope.

"The blue and white and several kinds of hairy mouldy spots.....are all of them nothing else but several kinds of small and variously figur'd Mushrooms, .....which will not be unworthy of our serious speculation and examination as I shall by and by shew."

**Anton van Leeuwenhoek**: Bacteria identified by microscopy in the 1670s

**Louis Pasteur**: (19th century) linked bacteria with disease

**Joseph Lister**: a proponent of “germ theory of disease” – This Edinburgh surgeon used carbolic soap (containing phenol) to prevent infections during surgery.

**Robert Koch**: identified micro-organisms for tuberculosis, cholera and typhoid

**Paul Ehrlich**: The father of modern chemotherapy. He used chemicals against infection and was the originator of the “Magic bullet” theory. He developed the first fully synthetic drug ‘salvarsan’ containing arsenic (1910). It was not very good against bacteria but used for sleeping sickness (protozoa) and syphilis (spirochaete disease).
NON SPECIFIC ANTIMICROBIAL AGENTS

Most early preparations to treat infections were based upon non-specific antimicrobial agents which were often strong irritants as well as being toxic to the patient! Many non-specific antimicrobial agents are still in use today and are generally classified according to their use. Some modern and recently used compounds are given below.

**Disinfectants:** Formulations where the agent is too toxic or corrosive for topical use. Limited to inanimate objects (sinks, toilets, floors)

- Bleach (NaOCl) e.g. Domestos, "kills all known germs dead"
- Hydrogen peroxide (H₂O₂)
- N-chloro compounds (slow release of chlorine, for use in swimming pools)

**Antiseptics:** Formulations that can be safely applied to the skin (topical use)

- *Iodine*: a “tincture” (ethanolic solution) was used for wound cleansing in 19th and early 20th century.

  *Potassium iodide:* Reports from as early as 1903 mention the use of potassium iodide as an antifungal agent. It is only effective for the treatment of sporotrichosis (caused by *Sporotrichum schenckii*), although some rare S.E. Asian and African fungi are susceptible. An oral dosing of 1 ml (three times a day) of a 1g/ml solution was slowly raised to 12-15 ml. This was continued for up to six weeks after disappearance of the lesions. Common side effects noticed were cold symptoms, acne and general gastro-intestinal disturbance, which were severe enough to require temporary reduction of dosing. The mode of action was unknown until recently, but a 1985 report claimed that the therapeutic effect was mediated through the direct action of iodine. A solution of 0.02 mg/ml was shown to kill *Sporotrichum schenckii* in 10 minutes.

**Phenols / Alkylhalophenols:**

- Aq. phenol from time of Lister – too corrosive and toxic.
- 4-Chloro-3,5-dimethylphenol is the active ingredient of Dettol and similar antiseptics.
- Thymol (2-isopropyl-5-methylphenol) an antifungal agent, which was applied as a dusting powder for superficial infections now only found as a general antimicrobial agent used in mouthwashes.
- Chlorothymol (4-chloro-2-isopropyl-5-methylphenol) more potent, but severely irritating to the mucous membranes.
- Hexachlorophene – toothpastes / deodorants. Now banned due to CNS damage
- Triclosan – modern toothpastes etc.
Benzoic/salicylic acids and other hydroxybenzoic acids are general antimicrobial agents, e.g. "Whitfield's ointment", a mixture of 6% benzoic and 3% salicylic acids, was used a topical treatment for ringworm (tinea) infections. Can be irritating on tender skin and so was diluted with an emulsifier.

**Quaternary ammonium salts (cationic surfactants)**

- Chlorhexidine gluconate, a guanidine derivative

![Chlorhexidine (Hibitane)](image)

- Cetrimide or CTAB (C_{16}H_{33}NMe_{3}Br)
- Bradosol®, PhOCH₂CH₃⁺N(Me)₂CH₂(CH₂)₁₀CH₃.Br⁻ has some activity against *Candida* species. It is still available as an antiseptic in throat lozenges, but the British National Formulary (Sept. 1997) states that "there is no convincing evidence that antiseptic lozenges.....have a beneficial action, and they sometimes irritate and cause sore tongue and sore lips".
- Roccal®, PhCH₂⁺N(Me)₂CH₂(CH₂)ₙCH₃.Br⁻, is used as a general disinfecting agent for pre-operative skin preparation. At one time recommended for superficial skin infections.

**Preservatives:**

Additives used in food and pharmaceutical products, as well as biological specimens, to prevent biodeterioration (by bacterial action or growth)

- *NaCl* – salted food products used since ancient times
- *Nisin* (34 residue polypeptide from *Streptococcus lactis*)
- *Mercury compounds:*
  They were highly toxic to bacteria, plants, fungi and animals. Mercuric salts, e.g. HgCl₂, are severe irritants as well. Organo mercury compounds were found to be less irritating, but still toxic. Examples include penetrane and thimersol

![Merthiolate (thiomersal)](image)

These were used as 'wound disinfectants' in a 0.1% solution. Organomercury compounds still had limited uses up to the 1950s as plant fungicides and for the preservation of leather, textiles and timber. Obviously not used widely nowadays due to the toxicity of the mercury.

- *Formaldehyde* – biological tissue samples etc. Aqueous formaldehyde is called ‘formalin’.
ANTIBIOTICS AND ANTIBACTERIALS

Chemotherapy

Chemotherapy is "the use of chemicals (drugs) to prevent, control or eradicate disease/infection" i.e. applications in medicinal, veterinary and agricultural chemistry. Mycology is the specific term which is used for the study of mycoses, which are those fungal diseases affecting humans and animals.

Why study antimicrobial chemotherapy? Many bacteria are pathogens, i.e. agents of disease in man, animals and plants

Why Study Chemotherapy Further?

Chemotherapy has been spectacularly successful, i.e. the era of antibiotics, but the 'final solution' is not yet here:

'new' infectious agents, e.g.  
Legionella pneumophila (legionnaires disease)  
Helicobacter pylori (gastric ulcers)  
Borrelia burgdorferi (Lyme disease)

resurgent pathogens, e.g.  
Mycobacterium tuberculosis  
[still the prime killer, ~3 x 10^6 deaths p.a.]

multidrug resistance, e.g  
'Superbugs' such as Staphylococcus aureus and Enterococcus faecium,  
opportunistic Gram-negative pathogens  
[hospital-acquired (nosocomial) infections]

The emergence of many antibiotic-resistant strains of once-sensitive bacteria is a major theme of current research and scientific literature, and is regularly publicised in the media (TV, the press).

Some examples:
- New Scientist 1993 ['Superbug' epidemic sweeps Japan]
- Horizon programme on TV October 1997 [possible enlistment of antibacterial viruses]
- Daily Telegraph October 1997 [Ward closure due to spread of Klebsiella infection]
- The E. coli O157 outbreak in Scotland 1996-7 [butcher's shop in Wishaw]
Course Structure

A. To provide a broad overview of the diversity of antibacterial and antifungal agents with reference to:
   - chemical structure [mainly restricted to their general features]
   - origins [natural sources; some chemical syntheses]
   - type of action [bactericidal vs. bacteriostatic (inhibitory)]
   - applications [disinfectant; antiseptic; preservative; systemic use]
   - antibacterial spectrum [some illustrations]
   - site and mode of action [the target and how/why does it work?]
   - resistance [mechanisms; causes; cures?]

B. To examine in more detail a few agents, modes of action, and resistance mechanisms.

DNA RNA Enzymic proteins

\[\text{Drug} \quad \begin{array}{c}
\text{Degradation/modification} \\
\text{Inactivation by binding} \\
\text{Inhibition of biosynthesis}
\end{array}\]

Cell wall

\[\text{Drug} \quad \begin{array}{c}
\text{Disorganisation; loss of barrier/transport properties} \\
\text{Weakened; loss of mechanical support; cell lysis}
\end{array}\]

The Microbes

**Bacteria:** Prokaryotic (no nuclear membrane), no chlorophyll, cell wall in addition to cell membrane. Many bacteria can be assigned to one of two major groups, based on the Gram-staining reaction. The differences in cell-wall structure and composition account for the differential Gram reaction. We will concentrate on the cell wall both as a target and as a resistance factor.

**Fungi**

Eukaryotic (with cell nucleus). Much more like mammalian cells therefore more difficult to target. Fungi, also known as mycophyta, include yeasts, moulds and rusts. Can be made up of single cells (such as the yeasts) or composed of multi-cellular filaments which are called hyphae (e.g. with the dermatophytes).
Competitive Inhibitors (Anti-metabolites)
Mostly growth factor analogues of some sorts

Sulphonamides

Origins:
Domagk (1927) began a systematic investigation of gold compounds, acridines and azo dyes. He worked with strain of bacteria from a fatal case of septicaemia. He discovered the red diazo dye prontosil had antibacterial activity and low toxicity (mice). He performed a miraculous cure of a 10-month old baby with staphylococcal infection in 1933 and cured his daughter of same infection in 1935. Won the Nobel Prize in 1939.

![Prontosil rubrum](image1)

![sulphanilamide active metabolite](image2)

Target:
The active agent is sulphanilamide, which is an analogue of p-aminobenzoic acid (a bacterial growth factor used in the biosynthesis of folic acid - acquired by man through the diet).

![Example Sulphamethoxazole](image3)

![Sulphanilamide](image4)

![p-Aminobenzoic acid](image5)

Action:
Competitive antagonism with p-aminobenzoic acid shown in 1940 by Donald Woods (Oxford) Bacteriostatic action. Folate used as co-factor in many enzymes as a one carbon atom donor.
Spectrum of activity:
Early successes against streptococci and has broad spectrum but problems with side effects, and resistance. Has some residual applications mainly for urinary tract infections and veterinary medicine.

\[
\begin{align*}
\text{Sulfapyridine} & \quad \text{Sulfadimidine} & \quad \text{Generic structure} \\
\text{(sulfapyridine used by Winston Churchill suffering from pneumonia in 1943 North Africa)}
\end{align*}
\]

Cell Membrane Agents
Polypeptides

Structural features:
Various structural types (cyclic/linear); some with ester linkages also; some with fatty acyl groups; many cationic; D-amino acids commonly present.

Origins: Several (tyrocidin, gramicidin S, bacitracin, polymyxins) produced by spore-forming GPB (Bacillus spp.). Little systemic use because of toxic side-effects

Target: the cytoplasmic membrane

Action: (in most cases) bactericidal; disruption of the structure or function of the bacterial membranes (permeability barrier). Similarities between phospholipids in eubacterial and eukaryotic membranes mean selectivity is low and rarely specific enough for systemic use.

- Tyrocidins, *e.g.* Gramicidin S: cyclic decapeptides
- Enduracin A: cyclic ester, fatty acyl substituent, D-Orn and other unusual amino acids. Inhibits peptidoglycan biosynthesis (lipid-linked precursors accumulate). Not used clinically.
- Gramicidin A: Linear, hydrophobic pentadecapeptide modified at both termini by a formyl substituent (Fm) and amidation by ethanolamine (Etn). The major component (90%) is that shown below.

\[
\]

- Bacitracin: isolated from a wound infection (bacillus)-patient named Tracy! Active against GPB for topical use (*e.g.* colon surgery), but poor tolerance and stability. Cyclic and branched.
- Polymyxins: (1947) from Bacillus polymyxa. *MOST IMPORTANT CLINICALLY* Cyclic, cationic (5+), fatty acylated. Active against GNB, including *Pseudomonas aeruginosa.* Nephrotoxicity (kidney) limits systemic use, but still applications as topical agent. Used under close medial supervision
• Other small cationic peptides seem to contribute to natural anti-bacterial defences and one (Nisin) is used as a food preservative. This cationic, linear, amphiphilic peptide of 34 amino acids is produced by *Lactobacillus lactis* subspecies *lactis*. It is active as an ionophore against other Gram-positive bacteria. Approved by the WHO as a preservative in food.

• Other cationic peptides occur widely in Nature and many probably have a protective antibacterial function. Some examples: defensins - from mammalian phagocytic cells; magainins - from frog skin; melittin - from bee venom

**Effects on Nucleic Acids**

*Quinolones*

**Structural features:**
A major group of broad-spectrum 'antibiotics' of recent development based on quinoline ring. *Brief review: Chem. Brit. 28 (1992) 34-36*

**Mode of action:**
Inhibition of enzyme DNA gyrase, also called topoisomerase II, an enzyme involved in uncoiling super coiled DNA (normally 1000 µm coiled to 1 µm) prior to cell division. These have selective action on prokaryotes unlike many other drugs acting on DNA.

**Origins:**
Prototype drug: *nalidixic acid* (1962): active against Gram-negative bacteria; uses for urinary tract infections (unpleasant side effects; poor pharmacokinetic profile; resistance developed quickly)

![Nalidixic acid](image)

![Ciprofloxacin](image)

**Spectrum of activity:**
Fluorination at position 6 gives much better activity and broader spectrum. Many new analogues being developed with improved activity vs GPB.

**Notes:**
• *Ciprofloxacin* (UK market, 1987): effective against many multidrug-resistant bacteria; well tolerated; rapidly growing market; may be best broad-spectrum agent now available.
**Synthesis of Ciprofloxacin**

```
\begin{align*}
\text{F-Cl-Cl} & \xrightarrow{\text{Mg(OEt)}_2} \text{F-Cl-Cl-CH(CO_2Et)_2} \\
\text{F-Cl-Cl-CH(CO_2Et)_2} & \xrightarrow{\text{H}^+} \text{F-Cl-Cl} \\
\text{F-Cl-Cl-CH(CO_2Et)_2} & \xrightarrow{\text{HC(OEt)_3}} \text{F-Cl-Cl-CH_2CO_2Et} \\
\text{F-Cl-Cl-CH_2CO_2Et} & \xrightarrow{\text{Ac}_2\text{O}} \text{F-Cl-Cl} \\
\text{F-Cl-Cl-CH_2CO_2Et} & \xrightarrow{-\text{HCl}} \text{F-Cl-Cl-NH} \\
\text{F-Cl-Cl-NH} & \xrightarrow{\text{Base}} \text{F-Cl-Cl-N} \\
\text{F-Cl-Cl-N} & \xrightarrow{\text{H}^+} \text{F-Cl-Cl-N} \\
\text{F-Cl-Cl-N} & \xrightarrow{\text{HN}_2\text{NH}} \text{F-Cl-N} \\
\text{F-Cl-N} & \xrightarrow{\text{H}^+} \text{F-Cl-N} \\
\text{F-Cl-N} & \leftarrow \text{F-Cl-N} \\
\end{align*}
```

*Ciprofloxacin*
**Ansamycins**

**Structural features:**
Two groups of macrocycles incorporating aromatic residues: rifamycins and streptovaricins.

**Origin:**
Rifamycin isolated (1957) from a *Streptomycyes sp.*; >100 semi-synthetic derivatives.

**Target:**
mRNA biosynthesis

**Action:**
bactericidal, binding to RNA polymerase enzyme (involved in the synthesis of mRNA)

**Notes:**
- *Rifampicin (rifampin)*: semi-synthetic (the hydrazone side-chain), oral, active agent GBP and GNB as well as *Mycobacterium tuberculosis* (a major drug for this infection)

Rifampicin (rifampin)

[a major anti-TB drug]

Rifampicin is a member of the *rifamycin* group of antibiotics. The rifamycins and the related *streptovaricins* belong to the *ansamycin* group of non-peptidic, macroyclic antibiotics.
Protein Synthesis Inhibitors

(see Organic Chemistry, by T.W.G. Solomons and C. Fryhle, 7th Ed p. 1243-1350 for a basic introduction)

**Tetracyclines**

**Structural features:**
As the names suggests, four fused (C6) rings, one benzenoid, with variety of substituents and functions. Note enol tautomeric structure of $\beta$-diketone(s).

![Tetracycline structure](image)

Chlortetracycline (aureomycin) = $7$ chlorotetracycline, $R = H$
Oxytetracycline (terramycin) = $5$-hydroxytetracycline, $R = H$
Glycyclines, $R = Me_2NCH_2CONH$-

**Origin:**
First isolated (1948) from a *Streptomyces sp.*

**Target:**
protein biosynthesis

**Action:**
bacteriostatic, prevent binding of aminoacyl-t-RNA to the ribosomes

**Spectrum of activity:**
Broad spectrum (GPB, GNB and rickettsias), but with weaknesses (*Salmonella*, *Proteus*, *Pseudomonas*) and resistance increasingly common.

**Notes:**
- Examples: *Chlortetracycline*, *oxytetracycline* (also natural)
- Semi-synthetic tetracyclines also marketed (*e.g.*, *Doxycycline*), but declining interest
- Clinical use declining, though still choice therapy for infection of urinary and respiratory tracts, urethra, pelvis; Lyme disease (*Borrelia burgdorferi*); sexually-transmitted diseases.
- UK market (1991): No.5 Oxytetracycline
- World sales of tetracyclines (1995) $\$0.5$ billion (No. 5=, but falling)
- Avoid use in children (Ca chelation $\rightarrow$ yellow teeth)
- Semi-synthetic tetracyclines also marketed (*e.g.*, *doxycycline*), but declining interest
**Macrolides**

**Structural features:**
Macro cyclic esters (lactones) with additional functions (C=O, OH), alkyl substituents, and attached amino/branched sugars.

**Origin:**
First isolated (1952, erythromycins) from a *Streptomyces* sp. Now > 100 known

**Target:**
protein biosynthesis

**Action:**
bacteriostatic, premature release of incomplete peptide, still attached to t-RNA

**Spectrum of activity:**
Excellent against many GBP, including streptococci and staphylococci, also pathogenic *Neisseria, Legionella, Chlamydia, Mycoplasma*, but most GNB resistant. Treatment of respiratory, skin, genital tract infections.

Notes: Treatment of respiratory, skin, genital tract infections.
- **Erythromycins:** example given is Erythromycin A. 14-membered ring. Acid-labile (degradation in the stomach): protect, e.g. by encapsulation or as a prodrug derivative (e.g. an ester)
- **Azithromycin:** 15-membered ring incorporating N. Good stability, oral potency, and pharmacokinetics
- **Carbomycins and Spiromycins:** 16-membered ring compounds, both incorporate a disaccharide substituent.
- **Clarithromycin:** a top-20 drug (respiratory tract and skin infections by streptococci etc) UK market (1991): Nos. 4 and 6 Erythromycins. World sales of macrolides (1995) $1.5 billion (No. 4, rising slightly)
**Aminoglycosides**

**Structural features:**
Cationic sugar-cyclitol based antibiotics: hydroxylated, basic cyclohexane; amino sugars; branched sugars; 3 or 4 linked rings.

![Structural diagram](image)

**Origins:**
First example (streptomycin) isolated (Waksman 1943) from a *Streptomyces* sp., as are most other examples (gentamicin a major exception).

**Target:**
protein biosynthesis

**Action:**
bactericidal; complex, variable, and incompletely defined, but includes (i) electrostatic, self-promoted uptake, (ii) ATP-dependent transport across the cytoplasmic membrane, (iii) binding to ribosomes, inhibiting their function

**Spectrum of Activity:**

**Notes:**
- *Neomycins*: (1949), tetracyclic (including D-ribose), very active but toxic, so mainly topical use.
- *Kanamycins*: (1957), two amino sugars attached directly to the aminocyclitol, improved vs. GP ccci.
- *Gentamicins: from Micromonospora*, relatively toxic but often effective against *Pseudomonas aeruginosa* and other problem 'pseudomonads'
- *Tobramycin*: natural relative of kanamycins
- *Amikacin*: semi-synthetic derivative of kanamycin A (improved stability to inactivating enzymes)
- *Spectinomycin*: 3 fused rings, no amino sugar, bacteriostatic. Used against *Neisseria gonorrhoeae*
- Aminoglycosides remain significant antibiotics, but use declining. Not in UK top 10 (1991). World sales (1995) $0.5 billion (No. 5= and falling)
**Chloramphenicol**

**Structural features:**
Chlorinated nitro aromatic compound.

![Chemical structure of Chloramphenicol](image)

D-threo isomer only. Both centres are of R configuration.

**Origin:**
Originally (1950s) natural (*Streptomyces*) but now synthetic.

**Target:**
protein biosynthesis

**Action:**
bacteriostatic, binding to ribosomes, blocking peptidyltransferase activity

**Spectrum of activity:**
Broad spectrum similar to tetracyclines, oral, but serious side-effects.

**Notes:**
Current applications include bacterial meningitis, brain abscesses, conjunctivitis, laboratory reagent.

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**Lincosamides**

**Structural features:**
Thioglycoside of Cs sugar with N-heterocycle (proline derivative) attached.

![Chemical structure of Lincosamides](image)

**Origin:**
*Lincomycin* obtained from *Streptomyces* sp. (1963); semi-synthetic chloro derivative (*Clindamycin*) has inverted configuration at C-7

**Target:**
protein biosynthesis
**Action:**
binding to ribosomes (similar site to erythromycin)

**Spectrum of activity:**
Active against many GPB, and also some anaerobic GNB (*Bacteroides* spp.). Clinical applications mainly the GN and GP anaerobes; colitis side-effect

**Streptogramins**

**Structural features / Origins:**
Two groups (A and B) structurally unrelated compounds, which occur as pairs in a *Streptomyces* sp.

Group A: polyunsaturated, macrocyclic lactones incorporating amide bonds.
Group B: cyclic hexadepsipeptides (peptide and lactone units) with aryl and other attachments

The combination of quinupristin with dalfopristin (both water-soluble, semi-synthetic streptogramins) has a synergistic, bactericidal action, mainly on Gram-positive cocci.

**Target:**
protein biosynthesis

**Action:** binding to ribosomes (similar site to erythromycin and lincosamides)

**Spectrum of activity:** Good activity against GPB but few GNB. Considered promising for MRSA.

**Notes:**
- Original (1961) pair (*Pristinamycins*) used to generate semi-synthetic, water-soluble, injectable streptogramins (see below) which are individually bacteriostatic but together synergistic and bactericidal.
- *Dalfopristin*, alias RP 54476, and *Quinupristin*, alias RP 57669, used as 7:3 mixture (*Synercid®, RP 59500 from Aventis*). Not yet licensed (Sept. 97) for clinical use in UK. Considered promising for MRSA and VRE (but see *Lancet* ref. below).

Cell Wall Biosynthesis Inhibitors

**β-Lactams**

**Structural features:**
All examples contain the 4-membered ring; a strained, cyclic amide (lactam) which is intrinsically labile to hydrolysis (*acidic or enzymatic*).

**Target:** cell-wall biosynthesis - more of this later

**Action:** bactericidal, active only against *growing cells*

**Some factors to consider regarding β-lactams:**

(a) They are inherently unstable towards acid due to the strained four-membered ring
(b) Many are unstable with regards enzymic degradation (susceptibility to β-lactamases)
(c) Their spectrum of activity may be quite variable

**PENAMS (Penicillins)**

![General penam ring structure](image)

**Structural features:** Four chiral centres, variable R group, and two consecutive amide bonds (one exocyclic, one endocyclic)

**Important examples (differing in R):**

- **Benzyl penicillin** (penicillin G)
- **Methicillin**
- **Ampicillin**
- **Amoxycillin**
- **Carbenicillin**

**Spectrum of activity:** *Penicillin G*, the prototype compound, remains valuable for aerobic GPB and some GNB (e.g. *pathogenic Neisseria*), but is unstable to acid and β–lactamases.

- **Methicillin:** more stable towards β-lactamases. Antibiotic of choice for *S. aureus*, but many hospital strains are now resistant (MRSA).
- **Ampicillin:** improved acid stability but degraded by staphylococcal β-lactamase. Broader spectrum of activity.
- **Amoxycillin:** improved absorption after oral administration. Used in conjunction with β-lactamase inhibitor (clavulanic acid) as *Augmentin*. 
Carbenicillin: effective against *Pseudomonas aeruginosa*

**UK market (1991): Nos. 1 and 2 Amoxycillins; No. 3 Penicillin G; No. 7 Augmentin; No. 10 Ampicillin.**

**Origins:** 1928 Fleming's lucky observation of antibacterial activity of product from a *Penicillium* mould.

Variation in R: ‘Pen G’ (R = PhCH$_2$) was isolated from *P. notatum* by Fleming. *P. chrysogenum* produced greater yield of pen G and production can be enhanced by addition of phenylacetic acid to the broth. It is unstable to acid therefore administered by injection only (due to acidity of gastric juices).

![Chemical structure of Pen G and Pen V](image)

‘Pen V’ (R = PhOCH$_2$) is also natural, and production can be increased by addition of the corresponding acid to the broth. It is more acid stable and can be taken by mouth.

Pen-G and Pen-V can be used against GPB but not GNB.

**Original Research Aim:** increase spectrum of activity and acid stability. Variation (in RCO-) for penams was initially achieved by supplementation of the fermentation with acyl precursors, but this doesn’t work for all acids.

Later, widespread use led to rapid emergence of resistance - esp. *Staphylococcus aureus* [Why? Bacteria produce β-lactamases (penicillinase) that hydrolyse the β-lactam ring– more later]

How to vary R to get more stable penicillins? (to both enzyme and acid). NB β-Lactam ring is much less stable than the exocyclic amide!

Later variants (~1959 onwards) obtained by chemical acylation of 6-aminopenicillanic acid (6-APA) after removal of the natural acyl group with a bacterial enzyme (Beecham's).

\[
\begin{align*}
\text{RCO$_2$H} + 6\text{-APA} & \rightleftharpoons \text{RCO-APA} + \text{H$_2$O} \\
\text{pH} = 5 & \text{pH} = 8
\end{align*}
\]

Leads to semi-synthetic penicillins, e.g. methicillin, ampicillin (1962) and amoxycillin (1971).
CEPHEMS (Cephalosporins)

General cephem ring structure

**Structural features:** Note fused 6-membered S-heterocycle (dihydrothiazine), 3 chiral centres, 2 variables ($R_1$, $R_2$). Also 3-CH$_2$OAc in Ceph C another site for modification.

**Important examples:**
- Cephalexin ($R_1$ as ampicillin, $R_2$ = H)
- Ceftazidime ($R_1$ as aztreonam, $R_2$ = pyridinium)

**Origins:** Isolation of the first cephalosporin from fungus *Cephalosporium* (1953). More recent (1970s) isolates (cephamycins) from *Streptomyces*

**Spectrum of activity:** Biological activity poor relative to pens, Improved resistance to acid, β-lactamases, broader spectrum of activity

- Semi-synthetic variants parallel the penicillins.
- *Cephalexin:* first generation. An alternative to penicillins vs. GPB.
- *Ceftazidime:* third generation. Much improved against GNB including *Pseudomonas aeruginosa*
- *Ceftriaxone:* third generation.
- UK market (1991): Not listed in top 10 but world sales of cephalosporins (1995) $6 billion and rising (No. 1)

- **Variants cannot** be obtained by adding acids to the broths.
- **7-ACA cannot** be produced enzymically – need a chemical method! How to hydrolyse the exocyclic amide and not the β-lactam??

**Chemical production of 7-ACA from cephalosporins**
Production from β-lactams? Aim to convert cheap Pen G in cephs.

CARBAPENAMS - Non-classical, natural β-lactams

**Structural features:** Note exocyclic S and absence of N-acyl substituent

**Origins:** 1970s Isolation of the prototype (thienamycin) from a new *Streptomyces*

**Spectrum of activity:** *Thienamycin*: natural, excellent vs. anaerobes (cf. other β-lactams), good against GPB (but easily hydrolysed). *Imipenem*: semi-synthetic, much more stable, clinically valuable.

- Unprecedented broad spectrum, (GNB, GPB)
- Not de-activated by β-lactamases
- but unstable ring system, limited pH range for stability (pH7) therefore used as iv formulation and high concentrations.
- Not possible to boost fermentation yields by strain selection, therefore difficult to obtain. Must be synthesised - a challenge (*Tetrahedron Letters*, 1980, 21, 2783).
MONOBACTAMS - Isolated in 1981 from a soil sample in New Jersey

\[
\begin{array}{c}
\text{General monobactam structure}
\end{array}
\]

**Structural features:** No fused rings, \( N \)-sulphonic acid.

\[
\begin{array}{c}
\text{Aztreonam}
\end{array}
\]

**Origins:** Isolated from bacteria

**Spectrum of activity:** *Aztreonam:* semi-synthetic. Active against GNB (mediocre vs. GPB)

Aztreonam (semi synthetic) Excellent chemical stability and resistance to \( \beta \)-lactamases. High potency towards GNB, and importantly strains of pseudomonad.

**GLYCOPEPTIDES**

**Structural features:**
Large, complex, polycyclic antibiotics incorporating amino acids and sugars, with other functions.

\[
\begin{array}{c}
\text{Branched-chain, 3-amino deoxysugar}
\end{array}
\]

Vancomycin
Also in clinical use: teicoplanin.
Other natural and semisynthetic glycopeptides are under development.
**Origins:** First and more important example (vancomycin) isolated (1956) from a *Streptomyces sp.*; ~100 members of the group, and much current interest.

**Spectrum of activity:**
Narrow spectrum of activities, but last line of defence (?) against some important or multidrug-resistant GPB. GPB only (too large to penetrate GNB)

- **Vancomycin:** from soil sample brought from Borneo by an American missionary. Rapidly put into clinical use (1959) for penicillin-allergic patients and -resistant *Staphylococcus aureus*. Use declined with arrival of methicillin, but 1980 → increased application as methicillin-resistant strains (MRSA) became prevalent. Alarm bells now ringing as vancomycin-resistant Enterococcus sp. (VRE) have emerged (1986 →) and resistance has been passed to *S. aureus* (1997 - in the laboratory only?)

- **Teicoplanin:** also natural and now marketed in the UK.

**Mode of Action of β-Lactams**

**Bacterial cell walls**
Fundamental differences in ultrastructure of the cell wall are responsible for the reaction (+ or -) of bacteria towards the Gram stain. In both types of cell, the *cytoplasmic membrane* is surrounded and supported by a *cell wall*, which provides strength, rigidity and shape. Schematic cross sections of these structures are given below.

- **Gram-positive**
  - Major component (~50%) is *peptidoglycan*

- **Gram-negative**
  - The cell envelope consists of a pair of membranes (*cytoplasmic* and *outer*) with a thin, intermediate layer of *peptidoglycan*

The glycan (polysaccharide) chain has pendant peptide side chains (of variable composition depending on the bacteria) that cross link to give extra rigidity and strength the cell wall.
Cross linking occurs through formation of amide bonds between chains, in some cases through a linking unit. The terminal D-Ala residue is lost as a “leaving group”.

**Key**

DAP, 2,6-diaminopimelic acid
Strominger hypothesis

Mode of Action of Glycopeptides

- The glycopeptide antibiotics (most notably vancomycin but also teicoplanin) inhibit late stages of peptidoglycan synthesis involving transfer of completed, lipid-bound precursor units from the cytoplasmic membrane to the growing cell wall.
- Inhibition occurs through H-bonding to the terminal dipeptide D-Ala-D-Ala.
- Glycopeptides are very large, complex molecules
- H-bonding to D-Ala-D-Ala involves 5 H-bonds but interaction is also facilitated by dimerisation (vancomycin) or the presence of a lipid anchor (teicoplanin)

High-affinity binding of glycopeptides via the "chelate effect" (cyclisation)

Structure-activity relationships for vancomycin and other glycopeptides (including the part played by dimerisation and hydrophobic anchors) are the subject of an extensive series of papers by Dudley Williams (Cambridge).
**Bacterial Resistance**

There are two general types of resistance: *intrinsic resistance* - inherent, natural, chromosomal and *acquired resistance* - resulting from alteration of the bacterial genome. Alteration of the bacterial genome can occur through:

**Vertical evolution:**
Mutation and selection is referred to as vertical evolution – Darwinian principles of natural selection.

**Horizontal evolution:**
Genetic transfer *via* plasmids or transposons (extra chromosomal elements) – can occur through various mechanisms.

Bacteria have various mechanisms by which they can exhibit or develop resistance to antibiotics:

- Enzymatic inactivation of the antibiotic
  e.g. β-lactams

- Modification of the target, becoming insensitive
  e.g glycopeptides

- Enhanced production of the target to compensate

- By-pass of the target, using an alternative insensitive route
  e.g. sulphonamides

- Exclusion of the antibiotic
  Generally for intrinsic resistance of GNB and of mycobacteria to many antibiotics *(e.g. of large or hydrophobic agents by many GNB)* [see below]

- Efflux mechanism to pump out the antibiotic
  e.g Tetracyclines, macrolides, quinolones, chloramphenicol

Some of these mechanisms have been considered in the course 'Lipids and Membranes' (06534), so we will consider here the case of bacterial resistance to β-lactams and glycopeptides.

Consideration of the mechanisms of bacterial resistance is almost as important as discovering new antibacterial agents. For example *S. aureus* is common and often harmless, but can cause infections ranging from boils and abscesses to meningitis and pneumonia: the leading cause of hospital (nosocomial) infections and deaths worldwide. A virulent epidemic MRSA (methicillin resistant *S. aureus*) strain has caused >1000 infections in Plymouth since April 1995 and it has been suggested that around 1 in 10 hospital patients will acquire an infection in hospital....*(not necessarily S. aureus)*
**Resistance to β-Lactams**

β-lactamases - enzyme inactivation of the antibiotic

β-Lactamases are enzymes that hydrolyse the cyclic amide bond of β-lactams and prevent them binding to PBPs (penicillin binding proteins, e.g. transpeptidases etc)

![β-Lactamase structure]

There are different sorts of β-lactamases:

for GPB:
- **inducible** (produced in response to a threat – economical for bacteria)
- **extracellular** (long-range action, but wastage by dilution)

for GNB:
- **cell-bound** (periplasmic space - strategic location)

What solutions are there to the β-lactamase problem?

- **Develop resistant β-lactams**
  
  *e.g.* methicillin: β-Lactamase resistance associated with increased steric hindrance about the α-C to the amide link. GPB produce basically one β-lactamase; GNB produce wide range of variants.

- **Inhibit the β-lactamases with another drug**
  
  *e.g.* by clavulanic acid (1976). This has little antibacterial activity but causes irreversible acylation of the β-lactamase enzymes.

![Clavulanic acid structure]

Clavulanic acid, isolated from *Streptomyces spp.*

Clavulanic acid comes from a *Streptomyces* species (1977); It use in combination with amoxycillin (1981) is a top-20 drug (called Augmentin®). Sulbactam is a further example. These ring systems are known as "clavams".
Resistance to Glycopeptides: modification of the target

Vancomycin is often the last resort for chemotherapy of *S. aureus* infections. This bacterium is common and often harmless but can cause a range of infections ranging from boils and abscesses to meningitis and pneumonia. It is the leading cause of hospital (nosocomial) infections worldwide.

**Vancomycin resistance:**
There is intrinsic resistance in GNB and some GPB (*e.g.* *Lactobacillus casei*, *Pediococcus pentosaceus*, *Leuconostoc mesenteroides*, *Enterococcus gallinarum*) but also acquired resistance in *Enterococcus faecium* (*‘VRE’ - considered as the problem of the 1990s, as charted in recent reviews*)

The prevalence of *E. faecium* and of VRE and multidrug resistant strains is rising. Acquired resistance in *S. aureus* is the nightmare scenario - a common fast growing bacterium with multidrug resistance. (1992 Laboratory transfer of vancomycin resistance from *E. faecalis* to *S. aureus*, *FEMS Microbiol. Lett.* 1992, **93**, 195)

**Mechanism of resistance of VRE to glycopeptides: an altered target**

High-level resistance to vancomycin (and teicoplanin) is acquired from a plasmid, resulting in the production of 7 new polypeptides, 3 of which confer resistance through the formation of a modified peptidoglycan precursor.

![Chemical diagram](image)

The replacement of Ala.Ala by Ala.Lac (*Lac = lactic acid where the NH₂ is replaced by an OH*) results in loss of one of the H-bonds critical for binding of vancomycin or teicoplanin, and affinity for the antibiotic decreases ~100x, and transglycosylation or transpeptidation are no longer inhibited.

The Lac residue is lost (as is the terminal Ala of the normal precursor) during cross-linking or through the action of a carboxypeptidase, and therefore is not found in the mature peptidoglycan.

Organisms with **intrinsic** resistance to glycopeptides have precursors similar or identical to X, explaining their resistance.
ANTIFUNGAL DRUGS
Fungi cause a range of illnesses (mycoses) ranging from the chronic to the serious. These mycoses can manifest themselves in a variety of ways. Infections can be superficial, that is situated at or close to the surface of the skin, or systemic which means they can affect the body as a whole, rather than individual parts or organs. A list of systemic infections is given in Appendix B.

Diseases such as athlete's foot (tinea pedis), 'jock' itch (tinea cruris), tinea manus (infection of the hand), thrush (oral and vaginal), and onychomycosis (affecting the nails) are examples of superficial infections caused by the dermatophytes from the *Trichphyton*, *Microsporum*, *Candida* (some can also cause systemic infections) and *Epidermophyton* species. 'Ringworm' (tinea corporis) is used as a general term for a fungal infection of the skin, in particular those of the scalp and feet. These infections are contagious, and cause intense itching. They are caused by one or more of these organisms together - classification is difficult as the diseases assume such a wide variety of forms - and similar symptoms can be caused by different organisms.

An important aspect to consider when developing treatments for mycoses is that fungi are eukaryotic. That is to say they have a nucleus within the cell containing the all important nucleic acids. In very simplistic terms this means that some of the biochemistry regulating fungi turns out to be very similar to animal cells. They are therefore unlike the prokaryotic bacteria which do not have a cell nucleus. This can in turn pose potential problems with toxicity. For many enzymes in a fungus there are related enzymes performing the same transformations in the human cell. If you want to target one of these enzymes with your drug then absolute potency may not be as important as the difference in potency of your drug towards the different forms of the enzyme.

### 5.ii AGENTS ACTING AGAINST THE FUNGAL CELL NUCLEUS

As we will see, most antifungal agents act against the cell wall steroid ergosterol, either by affecting its biosynthesis or through interaction with it when it is situated in the cell wall. However, there are two antifungal drugs that affect the fungal cell nucleus, namely griseofulvin and 5-fluorocytosine.

#### Griseofulvin

**History**

An unknown compound isolated (1947) from *Penicillium* cultures was shown to cause distortion of mycelial hyphae during growth of certain fungi. This isolate was named the 'curling factor' and was later shown to be the same as griseofulvin isolated in 1939. In 1958 Gentles *et al* reported in *Nature* the efficiency of griseofulvin in curing experimental ringworm infections in guinea pigs. Later that year, a report described its use in treating similar infections in man. Griseofulvin is a spiro-benzo[b]furan natural product, produced by *Penicillium griseofulvum*. It was isolated in 1939, but not fully characterized until a few years later. It is biosynthesized via the polyketide pathway, using a phenolic radical coupling to form the spiro ring (*c.f.* year 3 Heterocycles course).
Overview of the Biosynthesis of Griseofulvin

via the Polyketide Pathway

Me-CO-S-Enz

HCO₃⁻ BCCP, ATP

BCCP = biotin carboxyl carrier protein

Me-CO-S-Enz

Me-CO-S-Enz

Me-CO-S-Enz

Me-CO-S-Enz

Me-CO-S-Enz

Methylation is achieved by SAM (S-adenosyl methionine). SAM is nature's equivalent of methyl iodide

Cyclisation is achieved by aldol condensations

chain extension by multiple additions of S-malonyl Co-A

Spectrum of Activity

Antifungal activity was shown to be present with mycelial fungi only. No activity against yeasts was observed. Griseofulvin shows fungiastatic activity against actively growing dermatophytes. It is only of use for treating chronic infections caused by these fungi, i.e. ringworm infections \( tinea pedis \) (athlete's foot), \( tinea capitis \) (an infection of the scalp), and
other similar skin/nail infections]. These infections are often caused by more than one genus of fungi, especially those from the *Epidermatophyton*, *Microsporum* and *Trichophyton* groups.

**Administration**
Griseofulvin is orally active, but topically inactive (it's unusual in this therapeutic area to have to have an orally active drug for a solely superficial infection). Normal administration is two 0.25-0.5g doses per day (for an adult). MICs for dermatophytes are typically 0.14-0.6 mg/ml. All dermatophytes are sensitive, but certain infections present more of a therapeutic problem than others do. Courses of therapy 2-3 weeks for uncomplicated infections, but toe-nail infections can last up to twelve months! Some people are naturally poor absorbers of the drug, and always have low blood level of the drug at all times. Courses of treatment are therefore prolonged.

**Side effects**
These are common-place but rarely serious. Headache and nausea are most frequent, abdominal pain, diarrhoea and vomiting less so. There is evidence that griseofulvin is teratogenic, causing abnormalities in mice foetuses. Hence pregnancy is a contraindication for this drug.

**Mode of Action**
Griseofulvin appears to arrest mitosis (cell division) in the metaphase (mode of action is not known precisely). This observation is consistent with the drug's efficacy against actively growing fungi only. Microtubules, or spindles, are essential cell structures for physical separation of the chromosomes within the dividing cell nucleus. They are polymers of a protein called tubulin (the "monomer"). Griseofulvin has been observed to cause spindle disorientation and chromosome scattering in the anaphase. Anti-tumour agents such as colchicine and the vinca alkaloids cause de-polymerisation of the microtubules by binding to receptors on the tubulin monomer. These are therefore known as spindle poisons and effectively arrest mitosis by breaking up the microtubules. It is proposed that griseofulvin acts slightly differently by altering the function of intact microtubule rather than causing them to de-polymerise. Only at higher concentrations is destruction of the microtubules observed.

**Miscellaneous**
Only the natural (+)-enantiomer is active, the (-) is completely inactive. Many analogues have been made and this has resulted in drugs of increasing water solubility (therefore greater absorption potential). Most analogues showed little or no improvement in activity against *Microsporum gypseum* when compared to griseofulvin. It is still available in the UK as Fulcin® (Zeneca) and Grisovin® (GlaxoWellcome).

**5-Fluorocytosine**

**History**
5-Fluorocytosine (5-FC) is a synthetic pyrimidine first made in 1957 as a prospective antitumour agent (*J. Am. Chem. Soc.*, 1957, 79, 4559). It can be considered as an analogue of the natural pyrimidine cytosine. It showed no antitumour activity, but its anti-fungal activity was observed in 1963 during a random screening programme.
Synthesis of 5-Fluorocytosine

\[
\begin{align*}
\text{MeO} & \text{O} \\
\text{H} & \text{O} \\
\text{EtO} & \text{CO} \\
\text{FH}_2\text{C} & \text{EtOK} \\
\text{EtO} & \text{CO} \\
\text{O} & \text{K}^+ \\
\text{NaOEt} & \text{EtOH} \\
\text{NH}_2 & \text{Br} \\
\text{EtS} & \text{NH}_2 \\
\text{POCl}_3 & \\
\text{Cl} & \text{F} \\
\text{EtS} & \\
\text{NH}_2 & \text{Cl} \\
\text{EtS} & \\
\text{aq. HBr} & \\
\text{NH}_2 & \text{Cl} \\
\end{align*}
\]

Spectrum of Activity

In 1964 5-FC was shown to be active \textit{in vivo} (mice) against experimental cryptococcal and candidal (yeast) infections.

<table>
<thead>
<tr>
<th>MARKED ACTIVITY</th>
<th>MODERATE ACTIVITY</th>
<th>NO ACTIVITY</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pathogenic yeasts, e.g. \textit{Candida albicans}, \textit{Cryptococcus neoformans} \textit{Torulopsis glabrata}</td>
<td>Some strains of \textit{Aspergillus}, \textit{Sporotrichum schenckii} \textit{Blastomyces dermatitidis}</td>
<td>\textit{Histoplasma capsulatum}, \textit{Coccidiodes immitis} \textit{Dermatophytic fungi}</td>
</tr>
<tr>
<td>MICs &lt;1-10 µg/ml</td>
<td>MICs 100-1000 µg/ml</td>
<td>MICs &gt;&gt;1000 µg/ml</td>
</tr>
</tbody>
</table>
Its spectrum of activity is narrow, is limited to yeast infections, and even so some 8-10% of *Candida* strains are resistant. The rapid de-novo resistance occurring during therapy has effectively limited its use in the treatment of candidal infections, and is a major drawback for this compound.

**Administration**

5-FC is quite hydrophilic. It can be taken orally, and high serum concentrations achieved. A 2g dose gives peak serum levels after 2-4 h, and the half-life is 4h. Cerebro-spinal fluid (CSF) levels are ca. 75% of serum levels. The drug is hardly subjected to any metabolism and 90% of a given dose is excreted unchanged in the urine. This makes it good for infections of the urinary tract! On the whole the drug is well tolerated and of low toxicity (the record held for the most 5-FC taken by a single patient is 10.7 kg over three years. The infection in this case was a serious systemic cryptococcal infection). The recommended dose is 150mg/kg every 6h.

**Side effects**

On the whole 5-FC is not a toxic drug and is well tolerated. Side effects are mainly due to the long courses of therapy. They include gastrointestinal (GI) disorders, nausea and diarrhoea, and in serious cases ulceration and enterocolitis. Excretion occurs through the kidneys, so renal function is sometimes impaired with extended dosing.

**Mode of Action**

5-FC shows both fungiastatic and fungicidal (higher concentrations) activity against yeasts. Only fungiastatic activity was observed against *Aspergillus fumigatus* implying multiple modes of action.

1. 5-FC enters the cell with the aid of the enzyme cytosine permease.
2. 5-FC is deaminated with cytosine deaminase to give 5-FU inside the cell.
3. 5-FUdRMP stops DNA synthesis *via* inhibition of thymidylate synthetase (see below) so no thymidine can be made from uracil.
4. 5-FURTP, in place of URTP, is incorporated in to RNA which then produces abnormal proteins. Levels of replacement of URTP by 5-FURTP can be up to 50%
5. Cytosine can be considered as an antagonist of 5-FC. *In vitro* tests show that addition of cytosine reverses the inhibitory effect of 5-FC.

(5-FC = 5-fluorocytosine, 5-FU = 5-fluorouracil, 5-FUdRMP = 5-fluoro deoxyuridine monophosphate 5-FURTP = 5-fluorouridine triphosphate)

It is not certain where the fungicidal or fungiastatic activities lie, or if the two mechanisms are linked in any way.
The activity of the permease and deaminase enzymes are believed to be an important factor in determining the spectrum of activity of 5-FC as well as incidences of secondary resistance. 5-FU cannot be used as the drug as permease activity for uracil is low. Uptake into the fungal cell is therefore poor. The GI disturbance noticed by some patients is believed to be due to conversion of 5-FC to 5-fluorouracil in the gut by a deaminase enzyme from gut bacteria. The low activity of permease enzymes in humans has been attributed to the relatively low toxic side effects of 5-FC. Co-therapy, or combination therapy, can be used to help the problem of the development of resistant strains. This strategy in particular was used for cases of cryptococcal meningitis (caused by Cryptococcus neoformans) where 5-FC was administered with Amphotericin B (see section 5.1). Clinical trials came in 1967, and it was marketed in the USA as Ancobon® in 1972. It is available in the UK as Alcobon® (Roche), a formulation for intravenous use.

Miscellaneous
Sterols (steroid alcohols) are important natural products found widely in nature. Although very similar, there are distinct differences between mammalian, fungal and plant sterols. Examples are cholesterol (found in mammalian cells), sitosterol (plant cells), and ergosterol (fungal cells). Cholesterol is vital for the production of hormones, vitamin D, bile salts etc in mammalian cells. Similarly, ergosterol is a sterol of major importance in fungi, though not necessarily with the same functions as cholesterol. Ergosterol was so named as it was first isolated from a fungus that also produced the ‘ergot’ alkaloids (though there is no relationship between the alkaloids and sterol beyond the name). Ergosterol was also isolated from baker’s yeast in 1926, and the amounts recovered were found to vary in quantity depending on cultivation conditions and methods. Typically, yeasts produce 0.1-2% ergosterol by dry cell mass, but can be as high as 10%.

**Role in cell wall structures**

Ergosterol is found mainly in the cellular membranes and plays a role in permeability regulation of the membranes. In the bi-layer structure of the membrane (see below), ergosterol forms clusters within the phospholipid layers and ergosterol is essential for viable, healthy fungal cells. In a key experiment (*J. Biol. Chem.*, 1978, 253, 6218) cultures of baker’s yeast were placed under anaerobic conditions. Without oxygen the biosynthesis of sterols is not possible (see squalene epoxidase below). Next, various sterols were added to see how cell growth progressed - i.e. replacing the *de novo* source of sterols with a variety of ‘added’ sterols.

- With ergosterol added normal growth was observed (compared to a control),
- With added cholesterol, growth was 23% of the control,
- With lanosterol (a common biosynthetic precursor to both cholesterol and ergosterol) <1% of normal growth was observed after a period of 72h.
- A more detailed study, along the same lines, revealed that the side chain of ergosterol was also important for normal cell growth and function.
- The biggest differences between the structure of lanosterol and ergosterol are the additional C-4 dimethyl and C-14 methyl substituents.

The conclusions drawn from this experiment are that ergosterol is essential for normal growth and function of cell walls and hence the viability of fungal cells. If one were able to interfere with the normal functioning of ergosterol, or deprive the fungal cells of this sterol, then we'd effectively be able to control fungal growth and maybe even kill the fungal cell.
Biosynthesis and key target enzymes

A host of antifungal drugs act by inhibiting various enzymes along the biosynthetic pathway to ergosterol. This pathway to steroids in general is called the mevalonate pathway. Key steps are (in relationship to drugs in this course):

- Squalene epoxidation (thiocarbamates, allylamines)
- Δ14 reduction and Δ8-Δ7-isomerisation (morpholines)
- C14 demethylation (azoles) (see section 5.v for more details)
5.iv AGENTS ACTING AGAINST THE
FUNGAL CELL WALL

The polyenes are a group of natural products isolated from micro-organisms of the genus Streptomyces. There are around 200 or so examples isolated to date, but many are either uncharacterized or only partially so. Most of the so-called polyene antifungal drugs are macrocyclic lactones with ring sizes varying from 12 to 37 atoms in size, though an exception is found in ambrucitin.

**Polyene antibiotics: Macrocyclic lactones**
*(Amphotericin and Nystatin)*

**History**
Nystatin was discovered in the labs of the New York State INstitute for health in 1951, and was originally known as fungicidin. Amphotericin B was isolated from Venezuelan river-bed soil samples in 1956. Its name arises from its amphoteric nature as it possesses an amino and a carboxylic acid function.

**Spectrum of Activity**
The polyenes are active against all sterol containing organisms *viz* yeasts, algae, protozoa, flatworms, filamentous fungi.

**Administration**
The polyenes are very poorly absorbed from the gut. Administration of Amphotericin B is
achieved in an injectable formulation, and is the only polyene that can be used so. Amphotericin is highly protein-bound in the blood. Formulations of Amphotericin B with lipids, such as Amphocil® (sodium cholesteryl sulphate) and Abecelet® increase solubility, stability and absorption of the drug. A formulation of Amphotericin B encapsulated in liposomes (AmBiosome®) is apparently significantly less toxic than the parent compound. Nystatin is too toxic for systemic use, but can be prescribed in a cream for topical use against candidal infections [e.g. Nystaform® or Nystan®]. As Nystatin is poorly absorbed from the gut, it can be prescribed as a suspension for oral administration against intestinal candidiasis.

**Side effects**
The polyenes are amongst the most effective antifungal drugs, yet also the most toxic. At best the patient receiving treatment for a systemic infection can suffer from cramps, chills and nausea. Renal failure is a potential problem, and this situation needs to be closely monitored and the dose regulated appropriately.

**Mode of Action**
Several polyene molecules associate with ergosterol 'clusters' in the fungal cell wall (see below). The **amphipathic** nature of the rod-like molecules mean that the lipophilic side of the molecule associates with the ergosterol steroid skeleton with the hydrophilic hydroxy side of the molecule pointing "inwards". This creates an ion-channel through which deregulated loss of ions such as K⁺ can occur. Binding of the polyenes to other similar sterols such as cholesterol can occur, therefore "explaining" the toxicity of these drugs. A general rule of thumb is that the molecules with the fewer double bonds are more toxic than those with more. So Amphotericin B and Nystatin can be used for treating human mycoses, but examples such as Filipin are far too toxic. In this latter case, gross disruption of the plasma membrane is observed, releasing low molecular weight materials and even small proteins.

![Filipin](image)

**Miscellaneous**
Polyenes possess three to eight conjugated double bonds, leading to reference to these molecules as "tetrabenzenes", "pentaenes" etc. They show characteristic UV-vis spectra due to these conjugated double bonds and changes in the UV-vis spectra are noticeable in the presence of sterols. Relative "binding affinities" of polyenes to various sterols could therefore be estimated. One side of the rod-shaped molecules is replete with hydroxy and oxy functions, the other with a lipophilic alkene structure. The molecules are therefore amphipathic. Amphotericin B is approx. 2.1nm in length, similar to a phospholipid in the cell membrane and the common sugar moiety found in the polyenes is mycosamine, or 3-amino-3,6-dideoxymannose.
POLYENE-CELL WALL INTERACTIONS
mode of action of polyene antifungals

**Linear polyenes**
*(Ambrucitin)*

**History**

Ambrucitin is not a macrocyclic lactone, like the others, but contains cyclopropane, pyran and carboxylic acid functional groups. It is a drug which did not pass into clinical trials, and Ambrucitin is one of a group known as "orphan" drugs. Development costs were considered to be too great for a drug to treat rare infections, and so it was shelved and not taken further. Effective in acute, experimental coccidiomycosis, histoplasmosis and blastomycosis (i.e. serious systemic infections). It has limited activity against yeasts, in particular *Candida albicans*. Ambrucitin is active orally, unlike the other polyenes, but was also tested topically for experimental ringworm infections in guinea pigs.

![Hydrophilic ion channel diagram](image)
5.v AGENTS AFFECTING ERGOSTEROL BIOSYNTHESIS

Thiocarbamates
(Tolnaftate and Tolciclate)

History
The sulfonamides (R$_1$SO$_2$NHR$_2$ e.g. sulfadiazine) provided in the 1930s the first examples of broad spectrum antibacterials. During the search for new antibacterial agents, the thiocarbamates Ar$_1$OC(=S)NR$_2$R$_3$ were synthesized and tested (but were ineffective as antibacterials). Examples include tolnaftate (also known as Napthiomate N and sometimes under the trade name Tinactin®) and tolciclate.

Spectrum of Activity
The thiocarbamates were found to have "strong" and selective antifungal properties against Trichophyton spp. in vitro. They are ineffective against bacteria and other fungi in general. They are used for topical treatment of skin mycoses, e.g. athletes foot, but are not very effective against nail and scalp mycoses. However they are active against actively growing cells only.

Mode of Action
The thiocarbamates inhibit the squalene epoxidase, a key enzyme in the biosynthetic pathway of sterol production. (c.f. the allylamines - see below)

Structure Activity Relationships

<table>
<thead>
<tr>
<th>Substituent, R</th>
<th>Anti-trichophyton activity</th>
<th>Substitution Pattern</th>
<th>Anti-trichophyton activity</th>
</tr>
</thead>
<tbody>
<tr>
<td>H</td>
<td>higher</td>
<td>$\alpha$, $\alpha'$</td>
<td>none</td>
</tr>
<tr>
<td>Me</td>
<td>highest</td>
<td>$\alpha$, $\beta'$</td>
<td>high</td>
</tr>
<tr>
<td>OMe</td>
<td>high</td>
<td>$\beta$, $\alpha'$</td>
<td>none</td>
</tr>
<tr>
<td>OH</td>
<td>high</td>
<td>$\beta$, $\beta'$</td>
<td>none</td>
</tr>
<tr>
<td>Halogen</td>
<td>slight</td>
<td></td>
<td></td>
</tr>
<tr>
<td>COOH</td>
<td>slight</td>
<td></td>
<td></td>
</tr>
<tr>
<td>NO$_2$</td>
<td>none</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Synthesis of thiocarbamate antifungal drugs (1960s)

\[
\begin{align*}
\text{Ar}^1\text{OH} & \quad \xrightarrow{\text{NaH}} \quad \text{Ar}^1\text{O}^-\text{Na}^+ & \xrightarrow{\text{S} \equiv \text{CCl}_2} & \xrightarrow{\text{Ar}^2\text{NMe}} & \text{Me} \\
\text{Cl} & \quad \text{Cl} & \quad \text{Thiophosgene} & \quad \text{Thiocarbonyl diimidazole} & \quad \text{general structural motif of the thiocarbamate antifungal drugs}
\end{align*}
\]

**Napthiomate-N**  \( \text{Ar}^1 = 2\text{-napthyl}, \text{Ar}^2 = 1\text{-napthyl} \)

**Napthiomate-P (Tolnaftate)**  \( \text{Ar}^1 = 2\text{-napthyl}, \text{Ar}^2 = 3\text{-methylphenyl} \)

**Tolciclate**  \( \text{Ar}^1 = (\text{see below}), \text{Ar}^2 = 3\text{-methylphenyl} \)

**Synthesis of the phenolic portion of tolciclate via benzyne**

\[
\begin{align*}
\text{Br} & \quad \xrightarrow{\text{F}} & \text{F} & \quad \xrightarrow{\text{MgBr}} & \text{benzyne} & \quad \xrightarrow{\text{Diels-Alder}} & \text{H}_2/\text{Raney-Ni} & \quad \xrightarrow{\text{HNO}_3/\text{H}_2\text{SO}_4} & \quad \text{H}_3\text{PO}_4(\text{aq}) & \quad \text{H}_2/\text{Pd-C}
\end{align*}
\]

**Miscellaneous**

Commonly administered as a 1% solution or cream in polyethylene glycol for topical application. Also available as a dusting powder [e.g. Mycil®, Crookes Healthcare (Boots)]. The thiocarbamates, by today’s standards, are not considered to be very effective.
Allylamine derivatives

(Naftifine and Terbinafine)

History

The allylamine naftifine was fortuitously discovered during the search for drugs to treat CNS disorders (by Sandoz now Novartis). Although its structure is relatively simple it proved to be a novel compound. The general structural motif for all active compounds is a tertiary allylamine, hence the name.

Spectrum of Activity

Naftifine is topically active against dermatophytes and shows some activity against yeasts. Terbinafine is active against a wider range of fungi including Aspergillus, Histoplasma and Candida species. The tert-buty1 side chain of Terbinafine is essential for activity against these particular fungi. However, it is most active against dermatophytes. The primary activity of allylamines appears to be fungicidal.

Mode of Action

The allylamines inhibit the squalene epoxidase, a key enzyme in the biosynthetic pathway of sterol production. (c.f. the thiocarbamates). The fungicidal activity appears to be due to the intolerance of the fungal cell to increased squalene levels. Candida albicans can tolerate raised levels of squalene and show only fungiastatic susceptibility to allylamines.

Miscellaneous

A diminution of up to 75% of the normal ergosterol level has been observed in cells treated with allylamine antifungal compounds. Terbinafine is 2,000 times more active against fungal squalene epoxidase than rat liver squalene epoxidase, and 1,000 - 10,000 times more active against fungal squalene epoxidase than human liver squalene epoxidase. The dose per kg to effect the same cure rate in experimental guinea pig trichophyisis is 4-6 mg for terbinafine and 485 mg for naftifine. An advantage over the azoles is that the squalene epoxidase does not involve a cyt-P450 co-factor.

Naftifine is topically active. It is active orally at much higher doses, but to no discernible advantage. Terbinafine is active orally (100×s more active than naftifine), and is used to treat for ringworm and nail infections of the nail where oral administration is considered appropriate (Lamisil®, Novartis). Terbinafine can also be administered topically as a 0.5-1.0% cream. Terbinafine has been reported to cause gastro-intestinal disturbance and nausea when used orally, and in rare cases serious skin reactions can be triggered.
SYNTHETIC ROUTES TO ALLYLAMINES

R1NH2 O==CH==CHR2
 PhH, -H2O
 Dean Stark trap

R1N==CHR==CHR2
 NaBH4

R1NH==CHR==CHR2
 CH2O, NaBH4

Me
 NaFTIFINE

R1NH==CHR==CHR2

DIBAL-H
 PhMe
 40 °C

Me
 TERBINAfine

R1N=CHR==CHR2
 CuCl, dioxane, Δ

Me

R1NH==CHR==CHR2

R1N=CHR==CHR2

HCl, H2O
 EtOH
**Morpholine derivatives**  
*(Fenpropimorph and Amorolfine)*

**History**

The morpholines were discovered in the late 1960s as a spin-off from research into plant growth regulators. They were initially used as agricultural fungicides. Examples of the agricultural fungicides are tridemorph and dodemorph. Tridemorph was used to treat yellow rust in cereals, Dutch elm disease and the banana fungal disease sigatoka. Dodemorph was used to treat powdery mildews. The early morpholines were 4-alkyl cis-2,6-dimethylmorpholines. They were at first incorrectly believed to act in a similar fashion to the polyenes as the 4-alkyl group was most often a long or large-ring lipophilic alkyl group. These plant fungicides led the search for morpholines with applications in the field of human mycoses, via fenpropimorph (late 1970s) and culminating in the discovery of amorolfine in 1981 (brought to market later).

![Structures of FENPROPIMORPH, R= H and AMOROLFINE, R = Me](image)

**Spectrum of Activity**

Amorolfine and fenpropimorph are strong inhibitors of fungal pathogens in plants and humans. They are highly active against dermatophytes. Amorolfine also shows moderate activity against some yeasts and moulds.

**Mode of Action**

Morpholines act at multiple sites in the ergosterol biosynthetic pathway, each to a different degree depending on the example. The most cited sites of inhibition of these compounds are the Δ14 reductase and Δ8-Δ7 isomerase enzymes (see earlier). Fenpropimorph and amorolfine are about 100×s more active than tridemorph against the Δ14 reductase, perhaps indicating this enzyme's importance for use to treat human mycoses with this class of drugs.

**Miscellaneous**

The *cis* stereochemistry of the dimethyl morpholine is important. For fenpropimorph the *trans* dimethyl is about half as active as the *cis-dimethyl* isomer. The (+)(R) isomer is much less active than the (-)(S) isomer. Amorolfine is currently prescribed as Loceryl® (Roche) in cream (for tinea pedis or manus) or nail varnish (for onchomycosis) formulations. Topical use of amorolfine can lead to transient burning sensations, erythema and pruritus.
RACEMIC SYNTHESIS OF AMOROLFINE

\[
\text{CH}_3\text{CO} + \text{CH}_2\text{=CH}_2 \xrightarrow{\text{KOH-MeOH}} \text{CH}_3\text{CH}\text{=CHCO}
\]

ENANTIOSELECTIVE SYNTHESIS OF FENPROPIMORPH

\[
{^t\text{Bu}}\text{CH}_2\text{OH} \xrightarrow{\text{Pseudomonas}\ sp.\ Lipase} {^t\text{Bu}}\text{CH}_2\text{OH}
\]

\[
{^t\text{Bu}}\text{CH}_2\text{OH} \xrightarrow{\text{LiAlH}_4/\text{THF}} {^t\text{Bu}}\text{CH}_2\text{CH}_3
\]

\[
{^t\text{Bu}}\text{CH}_2\text{Cl} \xrightarrow{\text{PPh}_3\ (\text{Cl}_3\text{CO})_2\text{CO}} {^t\text{Bu}}\text{CH}_2\text{CH}_3
\]

\[
{^t\text{Bu}}\text{CH}_2\text{OH} \xrightarrow{\text{Tosyl chloride pyridine}} {^t\text{Bu}}\text{CH}_2\text{CH}_3\text{OTosyl}
\]

\[
{^t\text{Bu}}\text{CH}_2\text{CH}_3\text{OTosyl} \xrightarrow{\text{Tosyl chloride pyridine}} {^t\text{Bu}}\text{CH}_2\text{CH}_3\text{OAc}
\]
AZOLE DERIVATIVES

History
The original impetus in the discovery of azole antifungal drugs came from Wooley (1944). He found that benzimidazole inhibited the growth of various organisms, and the inhibitory effects were reversed by the addition of adenine or guanine (suggesting, wrongly, it was acting as a purine mimic). This observation led to the synthesis of 1-(p-chlorobenzyl)-2-methyl imidazole (known as chlormidazole, 1959), which had some activity against yeasts and dermatophytes. Many agricultural antifungal drugs were developed based upon the azole ring. In the late 1960's and early 1970's hundreds 1-substituted imidazoles were made, and a few were licensed for use in human therapy. Major companies working in this area were/are Bayer (Germany) and Janssen (Belgium), with Pfizer (US) and ICI/Zeneca (UK) following on.

HISTORY OF THE DEVELOPMENT OF THE AZOLE ANTIFUNGAL AGENTS

- **1969 (1972)** MICONAZOLE
- **1969** ISOCONAZOLE
- **1969** ECONAZOLE
  (Janssen)

- **1979** KETOCONAZOLE
  (Janssen)

- **1983/4** ITRACONAZOLE
  (Janssen)

- **1969** CLOTRIMAZOLE
  (Bayer)

- **1969** TIOCONAZOLE
  (Pfizer)

- **1975** (1979)

- **1979** FLUCONAZOLE
  *(diflucan)*
  (Pfizer)

- **1981/2** (1988)

- **1995** VORICONAZOLE
  (Pfizer)

- **1969 (1974)** BIFONAZOLE
  (Bayer)
  *ca. 1980*

Key
IMIDAZOLE or TRIAZOLE
DATES: DISCOVERY
(BROUGHT TO MARKET)
Mode of Action

The azoles, as a group of antifungal drugs, act by inhibiting the C-14 demethylation of sterols. This is a key step in the conversion of lanosterol to ergosterol. The demethylation is a multi-step, enzyme-mediated pathway involving a series of oxidation steps catalysed by the iron-haem protein cytochrome P-450 (J. Biol. Chem., 1984, 259, 1661). The azoles co-ordinate to, and therefore block, the haem iron atom (in Cyt-P450). This is a site normally occupied by O₂, and is a key part of the activation of molecular oxygen for the oxidation process that the enzyme mediates. All azoles are fungistatic at their MIC, but some are fungicidal at higher concentrations. Inhibition of these steps lead to a build up of C-14 methyl sterols, which replace ergosterol in the cell membrane. Ergosterol is quasi-planar and stabilizes the phospholipid membrane, but the C-14 methyl sterols are non-planar. This results in a series of changes in the fungal cell, e.g. change in cell membrane; an increase in cell volume; abnormalities in cell division and cell function.

Drugs that affect these enzymes can be selective to the fungal system (i.e. not affecting the biosynthesis of cholesterol in mammalian cells).

First generation azoles: imidazoles

Clotrimazole (Canestan®, Bayer, 1969), Miconazole (Daktarin®, Janssen, 1969)

Spectrum of Activity

Azoles possess very broad activity, with effects on:
- dermatophytes - Trichophyton, Microsporum, Epidermophyton;
- dimorphic fungi - *Histoplasma, Blastomyces, Coccidioides*;
- filamentous fungi (moulds) - *Aspergillus*;
- yeasts - *Candida, Cryptococcus* etc;
- some gram positive bacteria.

The anti-mycotic activity of the imidazoles was "enormous" compared to previous therapies. e.g. Compared to griseofulvin for treatment of athlete's foot, courses of therapy are drastically reduced (e.g. from 3 weeks to 1-3 days, with one dose/treatment per day instead of 2-4 doses).

\[
\begin{align*}
\text{CLOTRIMAZOLE} & \quad \text{MICONAZOLE} & \quad \text{ECONAZOLE} \\
\text{ISOCONAZOLE} & \quad \text{TIOCONAZOLE}
\end{align*}
\]

**Administration**

These early imidazoles were lipophilic. They have very poor aqueous solubility, so are generally ineffective for oral or systemic therapy. They have exceptional skin/mucous membrane compatibility and so are useful for topical application. They are normally formulated as creams for dermatophyte infections, or as a "nail varnish" formulations for onchomycosis. Competition to clotrimazole and miconazole, the 'big two', came in the form of econazole nitrate (*Ecostatin®, Bristol Myers Squibb* and *Pevaryl®, Janssen-Cilag, 1970s*), sulconazole nitrate (*Exelderm®, Zeneca*) and tioconazole (*Trosyl®, Pfizer, 1975*)

Miconazole provides an exception to the others in that it is absorbed in the gut slightly better. A gel formulation is available for oral and intestinal fungal infections. However miconazole binds strongly to lipoproteins - up to 95% is protein bound and only 20-30% of a dose is detectable in the blood. Its 'effective' concentration is therefore much reduced. Additionally the imidazole ring is subject to considerable **metabolic inactivation**.

**Miscellaneous**

These drugs are cheap and easy to make, so are still commonly prescribed even though they are nearly thirty years old. Incidences of secondary resistance were uncommon, but are now rising (1990s onwards). Potential problems with these drugs are minimal risk when used topically, but systemically used they have:

- Potential hepatotoxic effects in long term therapy
- Possible teratogen / embryotoxin effects
• May effect testosterone / corticosteroid synthesis (acting against other cytochrome P450 enzymes).

**Second generation imidazoles**

*(Ketoconazole, Nizoral®, Janssen, 1979)*

Janssen sought to improve upon miconazole and econazole, but retain the important advantages of the early imidazoles; namely high anti-mycotic activity and broad spectrum of activity. Their main aim was to provide a drug that was bio-available; i.e. that was more soluble in water and could be used for systemic infections either by injection or as a tablet.

Key stages in the development of ketoconazole were:

- The *imidazole* ring was found to be essential for activity; replacement with other related groups (e.g. benzimidazole) showed no activity.
- The *benzyl amines* showed some *in vitro* activity against dermatophytes.
- A common synthetic precursor to the amines and ethers were the phenacyl ketones which lead to the preparation of the *ketals*. These were not startling compounds. Basically, they had similar activity to miconazole both *in vitro* and *in vivo.*
**DEVELOPMENT OF KETOCONAZOLE**

- Chlorophenesin was known to possess anti-fungal activity and so this led to the preparation of the glycidyl ethers retaining the ketal group (dioxolane ring).
- It was observed that the cis isomer, with respect to the substituents in the dioxolane ring, showed superior activity in vitro and in vivo to the trans isomer.
- Significantly these compounds were as potent as the early imidazoles, retained the wide spectrum of activity and were much more soluble than miconazole so could be administered by mouth.

The disadvantages of ketoconazole:

- its efficacy against *Aspergillus* is limited;
it is metabolically vulnerable (<5% excreted unchanged);
95-99% of drug is bound to lipoproteins in serum (activity drops 10-1000 fold in the presence of serum with in vitro tests);
poor penetration into CSF;
best absorbed by gut in acid conditions, so antacids should not be prescribed at the same time as ketoconazole.

First generation triazoles

[Fluconazole (Diflucan®, Pfizer, 1981), Itraconazole (Sporanox®, Janssen, 1983); Voriconazole (Pfizer, 1995)]

Fluconazole

At the beginning of the 1970's the only antifungal drugs available for the treatment of systemic infections were Amphotericin B and Flucytosine. Pfizer sought to develop an antifungal drug that had: A broad spectrum of activity; could be administered by both oral and i.v. formulations (some cancer patients find oral dosing difficult and so availability of an i.v. formulation is important); that was metabolically stable and hydrophilic (to give good active concentrations of the drug); that crossed the blood-brain barrier, and of course was non-toxic (selective for an fungal enzyme/process). Key stages in the development of fluconazole were:

- The starting point was the newly discovered clotrimazole and miconazole, which lead to the discovery of tioconazole. [Tioconazole is available in a nail-varnish formulation, known as Trosyl®, for the treatment of onchomycosis.]
- During their search, the discovery of ketoconazole was announced but this was still metabolically vulnerable and bound to proteins (therefore "low active concentration").
- Focussing on possible structural variations the Pfizer team identified the tertiary alcohols as interesting targets as they should show increased hydophilicity compared to the ethers, and are structurally more distinct (and therefore more easily patentable).

Soon the imidazole ring was identified as the main problem with regards to the metabolic vulnerability; but with what could it be replaced? Of all the heterocycles tested the 1,2,4-triazole ring was the only one with which activity was retained. In comparison to the imidazoles, the triazolyl compounds showed reduced activity in vitro, but increased activity in vivo. This is because the triazole ring was far less readily metabolized. The bis-triazolyl compounds were the break-through point.
- The 2,4-dichlorophenyl bis-triazolyl candidate was stable in vivo. More than 30% was excreted un-metabolized (c.f. <1% for ketoconazole). However it was teratogenic in rat trials and showed hepatotoxicity in mice and dogs.
• All aryl bis-triazolyl variations gave active compounds, but only the 2,4-difluoro was not toxic. This difluoro compound was called fluconazole (Diflucan®).

**SEQUENCE OF FLUCONAZOLE DEVELOPMENT**

<table>
<thead>
<tr>
<th><strong>SPECTRUM OF ACTIVITY</strong></th>
<th>Excellent - only weakness against <em>Aspergillus</em> <em>sp.</em> (see Voriconazole, below)</th>
</tr>
</thead>
</table>
| **PROPERTIES**           | Water soluble (8 mg cm^2 at RT)  
Half life in serum 30 h - a 200 mg dose is still detectable ca. 7 days later (see below, Diflucan One®)  
Binds only weakly to blood proteins, therefore high 'active' concentrations in blood serum. |
Crosses blood-brain barrier well - CSF levels 60-80% that of blood serum.

**SELECTIVITY**

10,000 fold more active against the fungal C-14 demethylase compared to mammalian equivalent - better than ketoconazole.

**MICs**

- $\text{MIC}_{50}$ (fungal enzyme) ca. $10^{-8}$ M (fluconazole and ketoconazole)
- $\text{MIC}_{50}$ (mammalian enzyme) $10^{-7}$ M (fluconazole)
- $\text{MIC}_{50}$ (mammalian enzyme) $10^{-6}$ M (ketoconazole)

**ROUTE OF ADMINISTRATION**

Hydrophilic; systemically available.

>90% of oral dose absorbed from GI tract.

Oral and i.v. formulations available.

**STABILITY**

90% Of drug excreted in urine unchanged - metabolic stability.

**TOXICITY**

No evidence of hepatotoxicity nor teratogenicity.

<5 % of patients reported minor GI disturbance/ nausea.

---

Introduced in the UK in 1998, and the US in 1990, an estimated 4,000,000 people have taken Diflucan® in the first five years of being licensed. Newly introduced Diflucan One® is a one-tablet treatment of vaginal candidiasis. It relies on the exceptional half-life, where one 200 mg dose is detectable in the blood 7 days later. This period of time is enough to cure the infection in almost all cases. With cancer chemotherapy and organ transplant patients suffering from Candidal infections, a study showed 100% cure rates with a two-week course of Diflucan®. It also has beneficial prophylactic effects at lower doses.

**Itraconazole**

Itraconazole, Sporanox®, is Janssen-Cilag's triazole anti-fungal drug. It is prescribed for oropharyngeal and vulvovaginal candidaisis, as well as tinea infections. It is also prescribed for systemic infections, such as aspergillosis, candidaisis and cryptococcosis, where other antifungal drugs are inappropriate or ineffective. It is metabolized in the liver and so should not be prescribed to patients with a history of liver disease.

**Voriconazole**

Voriconazole is Pfizer's recently announced (1995) anti-fungal drug. It was developed to fill the gap in the armoury of Diflucan®, namely activity against Aspergillus infections. An extra methyl substituent was shown to be good for improved potency, but created stereocentres in the molecule and therefore four possible diastereoisomers. Almost all activity resides in the \textit{anti} ($R,S$)-isomer. The second triazole ring was replaced with a pyridine ring, and this was found to improve anti-Aspergillus activity, but proved to be metabolically vulnerable. The pyrimidine ring improved activity against \textit{Aspergillus} spp and together with the fluoro substituent improved metabolically stability.
DEVELOPMENT OF VORICONAZOLE

Lead structure: FLUCONAZOLE
Poor activity against emerging pathogens such as Aspergillus spp., and Candida krusei, but need to retain advantages of low toxicity etc.

![Chemical structure](image)

- Increased potency with methyl group. *Syn* and *anti* isomers possible.
- Pyridine group gave excellent aspergillus activity, but metabolically unstable.
- Better - but need to protect pyridine ring against metabolism by making more electron deficient.

VORICONAZOLE -
IC₅₀ 0.053 µM vs Aspergillus fumigatus
Almost all activity resides in (R,S) enantiomer shown

Fluoropyrimidine ring much more stable *in vivo.*
Diastereoisomers separated via chromatography.
Enantiomers via recrystallisation with camphor sulfonic acid.

SYNTHESIS OF VORICONAZOLE

![Chemical structure](image)

Lithium Disopropyl Amide a strong, non-nucleophilic base

anti

Voriconazole
### APPENDIX A: Bacterial Infections

<table>
<thead>
<tr>
<th>Organism</th>
<th>Disease/Infection</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Gram-positive cocci</strong></td>
<td></td>
</tr>
<tr>
<td>Streptococcus pyogenes</td>
<td>Tonsilitis, scarlet fever, impetigo, suppurative infections, rheumatic fever</td>
</tr>
<tr>
<td>Streptococcus pneumoniae</td>
<td>Pneumonia, sinusitis</td>
</tr>
<tr>
<td>Streptococcus mutans</td>
<td>Dental caries</td>
</tr>
<tr>
<td>Staphylococcus aureus</td>
<td>Miscellaneous suppurative infections, cystic fibrosis lungs</td>
</tr>
<tr>
<td><strong>Gram-positive bacilli</strong></td>
<td></td>
</tr>
<tr>
<td>Bacillus anthracis</td>
<td>Anthrax</td>
</tr>
<tr>
<td>Bacillus tetani</td>
<td>Tetanus</td>
</tr>
<tr>
<td>Clostridium welchii</td>
<td>Gas-gangrene</td>
</tr>
<tr>
<td>Clostridium botulinum</td>
<td>Botulism</td>
</tr>
<tr>
<td>Clostridium difficile</td>
<td>Hospital diarrhoea</td>
</tr>
<tr>
<td>Listeria monocytogenes</td>
<td>Listeriosis</td>
</tr>
<tr>
<td><strong>Gram-negative cocci</strong></td>
<td></td>
</tr>
<tr>
<td>Neisseria meningitidis</td>
<td>Meningitis</td>
</tr>
<tr>
<td>Neisseria gonorrhoeae</td>
<td>Gonorrhoea</td>
</tr>
<tr>
<td><strong>Gram-negative bacilli</strong></td>
<td></td>
</tr>
<tr>
<td>Salmonella typhi</td>
<td>Typhoid</td>
</tr>
<tr>
<td>Salmonella typhimurium</td>
<td>Food poisoning</td>
</tr>
<tr>
<td>Shigella dysenteriae</td>
<td>Bacillary dysentery</td>
</tr>
<tr>
<td>Vibrio cholerae</td>
<td>Cholera</td>
</tr>
<tr>
<td>Yersinia pestis</td>
<td>Bubonic plague</td>
</tr>
<tr>
<td>Bordetella pertussis</td>
<td>Whooping cough</td>
</tr>
<tr>
<td>Haemophilus influenzae</td>
<td>Meningitis</td>
</tr>
<tr>
<td>Brucella species</td>
<td>Brucellosis</td>
</tr>
<tr>
<td>Legionella pneumophila</td>
<td>Legionnaire’s disease</td>
</tr>
<tr>
<td>Helicobacter pylori</td>
<td>Gastric cancer (?)</td>
</tr>
<tr>
<td>Escherichia coli (0157!!), Serratia, Enterobacter, Proteus, Klebsiella, Acinetobacter, Pseudomonas, Burkholderia, and Stenotrophomonas spp.</td>
<td>Hospital-acquired (nosocomial) infections (“opportunistic pathogens” with multidrug resistance)</td>
</tr>
<tr>
<td><strong>Actinomycetes and related bacteria</strong></td>
<td></td>
</tr>
<tr>
<td>Corynebacterium diphtheriae</td>
<td>Diphtheria</td>
</tr>
<tr>
<td>Mycobacterium tuberculosis</td>
<td>TB</td>
</tr>
<tr>
<td>Mycobacterium leprae</td>
<td>Leprosy</td>
</tr>
<tr>
<td><strong>Spirochaetes</strong></td>
<td></td>
</tr>
<tr>
<td>Treponema pallidum</td>
<td>Syphilis</td>
</tr>
<tr>
<td>Leptospira species</td>
<td>Léptospirosis</td>
</tr>
<tr>
<td>Borrelia burgdorferi</td>
<td>Lyme disease (tick-borne)</td>
</tr>
<tr>
<td><strong>Rickettsias</strong></td>
<td></td>
</tr>
<tr>
<td>Rickettsia species</td>
<td>Typhus</td>
</tr>
<tr>
<td>Coxiella burnetii</td>
<td>Q fever</td>
</tr>
<tr>
<td>Chlamydia species</td>
<td>Trachoma, psittacosis, sexual diseases</td>
</tr>
</tbody>
</table>
APPENDIX B: Drugs to Treat Tuberculosis and Leprosy

_Mycobacterium tuberculosis_ and _mycobacterium leprae_ are the causative agents of TB and leprosy respectively. These bacteria are unique and different in many ways from most ‘common’ GPB and GNB. As a result individual drugs have been discovered/developed solely for the treatment of mycobacterial disease.

- **p-Aminosalicylic acid**: 1 of 3 major drugs for TB. Reason for specificity unknown; to be a salicylate antimetabolite not of _p_ -amino benzoate once believed. Doses can be ~10 g/day!! Unpleasant taste and causes gastric problems.

- **Dapsone**: the antileprosy drug (specificity again unclear); not a sulphonamide but a similar sulphone.
- **Pyrazinamide** - its mode of action may relate to inhibition of mycolic acid synthesis and the disruption of the cell wall in susceptible organisms.

- **Rifamycin and streptomycin** (see ansamycins, section 3.iii)
- **Ethambutol** (a hydrazine) - mode of action unknown, possibly due to interference with the biosynthesis of arabinogalactan, a cell wall polymer found in Mycobacteria but in few other bacteria.
- **Isoniazid** (a hydrazide) - seems to inhibit mycolic acid biosynthesis (a fatty acid associated with the outer cell membrane)
APPENDIX C: Systemic Fungal Infections

*Actinomycosis:* Caused by *Actinomyces bovis.* Common, world wide distribution. An infection of cattle transmissible to man, thought to be acquired endogenously. Symptoms vary - most often encountered are tumours in the jaws and tongue, which have hard woody lesions.

*Blastomycosis:* Caused by *Blastomyces dermatidis.* North American blastomycosis, common in central and eastern USA, is characterized by an initial pulmonary infection followed by progressive dissemination to other organs. A second form of the disease, common in other parts of the Americas, is a progressive disease of the mucous membranes, lungs, lymph nodes, skin and viscera.

*Nocardiosis:* Caused by *Nocardia asteroides* or *N. brasiliensis.* These have been found in soil, and are believed to be acquired by inhalation of dust containing the organism. Pulmonary infection may closely simulate tuberculosis or actinomycosis. The incidences of nocardiosis as a terminal condition of fatal diseases appears to be on the increase. A localized form of this disease, called mycetoma or Maduran foot, can eventually cause extensive deformities.

*Cryptococcosis:* Caused by *Cryptococcus neoformans.* World wide distribution of this saprophyte, believed to be an opportunistic pathogen taking advantage of defective defensive mechanisms in the host. Results in tumour-like lesions in the lungs, but may spread to the brain leading to meningitis.

*Sporotrichosis:* Caused by *Sporotrichum schenckii.* World wide distribution - most common in agricultural workers. *S. schenckii* is found in plants, trees and soil. The condition is characterized by multiple sub-cutaneous nodules along the course of the lymphatics, and infection is believed to be through infection of damaged skin or cuts.

*Histoplasmosis:* Caused by *Histoplasma capsulatum.* Once rare, now appears to be the one of the most widely distributed systemic mycoses. Causative organism is a small budding fungus, responsible for a variety of symptoms such as anaemia, loss of weight and irregular fever. Most cases are asymptomatic. Serious disseminated forms of this disease are most often found in infants or middle aged people with lymphomas particularly TB and Hodgkin's disease.

*Coccidiomycosis:* Caused by *Coccidioides immitis.* Endemic in arid and semi-arid areas. Found in the soil and acquired by direct inhalation of spores. The disease varies from slight non-specific pneumonitis to progressive and chronic granulomatous disease. The disease is asymptomatic or slight in about 60% of patients.

*Aspergillosis:* Caused by *Aspergillus fumigatus.* Usually a secondary infection of the lungs affecting devitalized areas of tissue in sufferers of diseases such as tuberculosis. Occupational health hazard of those working with straw, grain or flour which are liable to be contaminated with spores of the organism.
## APPENDIX D: Glossary of Terms

<table>
<thead>
<tr>
<th>Term</th>
<th>Definition</th>
</tr>
</thead>
<tbody>
<tr>
<td>Antifungal</td>
<td><em>see</em> antimycotic, fungicide and fungiastat.</td>
</tr>
<tr>
<td>Antimycotic</td>
<td>a drug active against fungi.</td>
</tr>
<tr>
<td>Aspergillus</td>
<td>a genus of fungi, which includes many common moulds, some of which cause infections of the respiratory system.</td>
</tr>
<tr>
<td>Candida</td>
<td>a genus of yeast-like fungi.</td>
</tr>
<tr>
<td>Candidiasis</td>
<td>(or candidosis) the general term for an infection with fungi of this genus.</td>
</tr>
<tr>
<td>Cryptococcus</td>
<td>a genus of unicellular yeast-like fungi, found in the soil (particularly when enriched with pigeon droppings), which are common in pigeon roosts and nests.</td>
</tr>
<tr>
<td>Dermatophyte</td>
<td>any microscopic fungus that grows on the skin or mucous membranes. The three main types are <em>Microsporum</em>, <em>Epidermophyton</em> and <em>Trichophyton</em>. These do not invade deeper tissues of the body.</td>
</tr>
<tr>
<td>Ergosterol</td>
<td>a plant sterol (steroid alcohol), which is an important feature of fungal cell walls.</td>
</tr>
<tr>
<td>Fungicide</td>
<td>an agent that kills fungi.</td>
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<tr>
<td>Fungiastat</td>
<td>an agent that inhibits or stops the growth of fungi.</td>
</tr>
<tr>
<td>Fungus</td>
<td><em>(pl. fungi)</em> a simple plant which lacks chlorophyll; includes yeasts, rusts, moulds and mushrooms. They live either as saprophytes or as parasites of plants and animals, and can cause disease (see mycosis).</td>
</tr>
<tr>
<td>Hypha</td>
<td><em>(pl. -e)</em> the filament of fungal plant body.</td>
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<tr>
<td>Mo(u)ld</td>
<td>a type of multi-cellular, parasitic filamentous fungus that commonly forms a rough furry coating (mycelium) on decaying matter. e.g. <em>Aspergillus</em></td>
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<tr>
<td>Mushroom</td>
<td>the aerial fruiting (spore producing) body of various fungi.</td>
</tr>
<tr>
<td>Myc-</td>
<td><em>[also myco-, mycet(o)-]</em> prefix denoting a fungus</td>
</tr>
<tr>
<td>Mycelium</td>
<td><em>(pl. mycelia)</em> the tangled mass of fine branching threads (filaments) that make up the feeding and growing part of a fungus.</td>
</tr>
<tr>
<td>Mycosis</td>
<td><em>(pl. mycoses)</em> any disease caused by a fungus, e.g. actinomycosis, aspergillosis, cryptococcosis, rhinosporidiosis, ringworm and sporotrichosis.</td>
</tr>
<tr>
<td>Parasite</td>
<td>an organism living in <em>(endo-)</em> or on <em>(ecto-)</em> another organism (host), from which it obtains food, yet contributes nothing to its welfare.</td>
</tr>
<tr>
<td>Parenteral</td>
<td>administered by any other route, other than by mouth <em>(per os).</em> Applied, for example, to the introduction of drugs by injection.</td>
</tr>
<tr>
<td>Phyt-</td>
<td><em>prefix denoting</em> plants; of plant origin.</td>
</tr>
<tr>
<td>Topical</td>
<td>local; used when referring to the route of administration of a drug applied directly to the part being treated (e.g. the skin or eye).</td>
</tr>
<tr>
<td>Saprophyte</td>
<td>an organism which lives on and feeds from dead and decaying tissues of plants and animals.</td>
</tr>
<tr>
<td>Superficial</td>
<td><em>(in anatomy)</em> situated at or close to the surface <em>(e.g. of the skin)</em></td>
</tr>
<tr>
<td>Systemic</td>
<td>relating to or affecting the body as a whole, rather than individual parts or organs.</td>
</tr>
<tr>
<td>Yeast</td>
<td>a unicellular fungus of the genus <em>Saccharomyces</em>. Important in brewing and baking, as well as being a commercial source of proteins and B vitamins.</td>
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</tbody>
</table>