Tetracyclines

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• Tetracyclines are antibiotics isolated from *Streptomyces aureofaciens* (aureo meaning golden colour).

• They are broad spectrum, act on Gram-positive, Gram-negative, aerobic and anaerobic bacteria.

• They bacteriostatic antibiotics.

• They inhibit protein synthesis by binding to the 30S subunit of ribosomes and preventing aminoacyl-tRNA from binding.

• They inhibit protein synthesis in bacteria and human cells, but penetrate faster to bacterial cells (which explain the selectivity).
• This family of antibiotics is characterized by a highly functionalized, partially reduced naphthacene (four linearly fused six-membered rings) ring system from which both the family name and numbering system are derived.

![Naphthacene](image1)

![Tetracycline](image2)

(1,2,3,4,4a,5,5a,6,11,11a,12,12a-dodecahydronaphthacene)
Several types of tetracyclines are in medical use such as:

1. Tetracycline
2. Chlorotetracycline
3. Oxytetracycline
4. Demeclocycline
5. Rolitetracycline
6. Meclocycline
7. Methacycline
8. Doxycycline
9. Minocycline

- **Natural**
- **Semisynthetic** (longer duration of action)
Tetracyclines are composed of four fused six-membered rings.

The structure have 5 or 6 chiral centres.

Tetracyclines are amphoteric compounds (i.e. form salts with either acids or bases)

Exist as insoluble zwitterions in neutral pH, which are dissolved by excess acid.

Formulated as HCl salt which is bitter in taste.

NaOH or KOH salts are less stable in aqueous solutions

Divalent and polyvalent salts are water-insoluble
• Tetracyclines acid salts have 3 acidity constants (i.e. pKa) in aqueous solution due to 3 protonation sites.

• The pKas for all types of tetracyclines are almost equal to: $pK_{a1}=3$, $pK_{a2}=7$ and $pK_{a3}=9$

• Have isoelectric point at pH 5 (at which zwitterionic form is dominated)

The conjugated phenolic enone system (C10 to C12) is neutral

The conjugated trione system (C1 to C3) is acidic

The moiety C-4-$\alpha$-dimethylamino is basic
Inactivation of Tetracyclines at neutral pH

- An interesting property of the tetracyclines is their ability to undergo epimerization at C-4 in solutions of intermediate pH range.

- These isomers are called *epitetracyclines* which are exist in equilibrium.

- The “epimer” exhibit much less activity than the “natural” isomers, thus accounting for the decreased therapeutic value of aged solutions.

- For this reason tetracyclines should be freshly prepared and used to gain the desired maximum activity.

![Chemical structures showing epimerization of tetracyclines](image_url)

**FIGURE 33.32** Epimerization of tetracyclines
Dehydration by Acids

Strong acids produce dehydration through a reaction involving the 6-hydroxyl group and the 5a-hydrogen. The double bond thus formed between positions 5a and 6 induces a shift in the position of the double bond between C-11a and C-12 to a position between C-11 and C-11a, forming the more energetically favored resonant system of the naphthalene group found in the inactive anhydrotetracyclines.

Inactivation of Tetracyclines by acids

Stable naphthalene group

Inactive anhydrotetracyclines
Inactivation of Tetracyclines by bases

- Bases promote a reaction between the 6-hydroxyl group and the ketone group at the 11-position, causing the bond between the 11 and 11a atoms to cleave, forming the lactone ring found in the inactive isotetracycline.

FIGURE 33.34  Base-catalyzed instability of tetracyclines.
Chemistry of tetracyclines (Cont.)

Dehydration by Acids

Inactivation of Tetracyclines by bases

Lactonization by Bases

Naphthalene

Inactive anhydrotetracyclines

Lactone

Inactive isotetracyclines
FIGURE 33.33  Acid-catalyzed instability of tetracyclines.
Tetracyclines inhibit protein synthesis by binding to ribosome at 30S subunit and possibly 50S subunit also.

There are more than one binding site for tetracyclines in ribosome.

Once the tetracycline binds, it inhibits subsequent binding of aminoacyltransfer-RNA to the ribosomes, resulting in termination of peptide chain growth.

The lipophilic tetracyclines (e.g. minocycline) are capable of disrupting cytoplasmic membrane also, thus having bacteriocidal properties.
• Tetracyclines inhibit protein synthesis by binding to ribosome at 30S subunit and possibly 50S subunit also.

• There are more than one binding site for tetracyclines in ribosome.

• All eastern-southern functional groups are involved in the interaction **EXCEPT** the dimethylamino group.

• The dimethylamino group is appeared not to interact with the ribosome but is still **ESSENTIAL** for activity.
FIGURE: Schematic representation of the primary binding site for a tetracycline and the sugar phosphate groups of 16S rRNA, which also involves a magnesium ion and the critical functional groups on the “southern” and “eastern” face of the tetracycline.
The key structural feature is the tetracycline skeleton
1. A linearly fused tetracyclic nucleus
2. and each ring needs to be six membered
3. and purely carbocyclic.

All derivatives containing fewer than four rings are inactive or nearly inactive.

The D-ring needs to be aromatic and the A-ring must be appropriately substituted at each of its carbon atoms for notable activity.

The B-ring and the C-ring tolerate certain substituent changes as long as the keto-enol systems (at C-11, 12, 12a) remain intact and conjugated to the phenolic D-ring.

The D, C, B-ring phenol, keto-enol system is important and the A-ring must also contain a conjugated keto enol system.
• Positions at the “bottom” of the molecule (10, 11, 1) and most of ring A (positions 2, 3, and 4) represent the invariant pharmacophore region of the molecule, where modifications are not tolerated without loss of antibiotic activity.

• In contrast, substituents at positions 5, 5a, 6, 7, 8, and 9, representing the largely hydrophobic “northern and western” faces of the molecule, can be modified with varying degrees of success, resulting in retention and, sometimes, improvement of antibiotic activity.
SAR for tetracyclines (Cont.)

Must be aromatic

1. Proper substitution at all C atoms
2. Keto-enol conjugated system
3. Tricarbonyl array at C1, C2 and C3
4. Basic amine at C4

- The enol-keto-enol conjugated system
- Conjugated rings
The keto-enol tautomerism of ring A in carbon atom 1 and 3 is a common feature to all biologically active tetracyclines, derivatives at C-1 and C-3 results **Blocking** this system and **loss of antibacterial activity**
C2-Carboxamide moiety is important for activity.

The amide is best left unsubstituted. N-monoalkylation results in prodrug which can be hydrolyzed in vivo to parent compound.

The replacement of carboxamide group even to nitrile (C≡N) or aldehyde reduces or abolishes the activity.

Larger alkyl group → disturb C1-C2-C3 tautomerism → reduce antibacterial activity e.g. Rolitetracycline is a prodrug which is more water soluble.
The α-hydrogen at C-4a position of tetracyclines is necessary for useful antibacterial activity. Should be preserved

The α-C-4 dimethyl amino substituent supports the keto-enolic character of the A-ring

Loss of activity occurs if dimethyl amino group is:
- having reversed stereochemistry
- being replaced with hydrazone (-N-N=C), oxime (-N-OH) or hydroxyl (-OH) groups.
Modification of the C-5 and C-5a positions:
- All natural active tetracyclines have unsubstituted C5 (i.e. R4=H)
- Alkylation of the C-5 hydroxyl group (i.e. R4=CH3) results in loss of activity.
- Substitution with C-5 α-hydroxyl group (i.e. R4=OH) give potent compound (e.g. oxytetracycline)
- Can be esterified to small alkyl esters to form semisynthetic tetracyclines which release oxtetracycline in vivo (prodrug).
- Epimerization (i.e. stereochemistry) is important for antibacterial activity. 5a-epitetracycline is inactive due to change in rings flatness

Activity is lost by:
- Double bond between 5a and 11a
- Aromatization of ring C
Modification at the C-6 position: mostly used to prepare semi-synthetic analogues
- The C-6 methyl group contributes little to the activity of tetracycline.
- The C-6 position is tolerant to a variety of substituents.
- Usually have $\alpha$-methyl group and $\alpha$-$\beta$-hydroxyl group at this position.
- Demeclocyclin is a naturally occurring C-6 $\alpha$-demethylated Chlortetracycline with an excellent activity.
- Removal of C-6 hydroxyl group affords doxycycline ($\alpha$-H, $\alpha$-$\beta$-CH3), which exerts good antibacterial activity, acid and base stability.
• 6-Deoxytetracyclines also possess important chemical and pharmacokinetic advantages over their 6-oxy counter parts. Unlike the latter, they are incapable of forming anhydrotetracyclines under acidic conditions because they cannot dehydrate at C-5a and C-6. They are also more stable in base because they do not readily undergo -ketone cleavage, followed by lactonization, to form isotetracyclines.

• Although it lacks a 6-hydroxyl group, methacycline shares the instability of the 6-oxytetracyclines in strongly acetic conditions. It suffers prototropic rearrangement to the anhydrotetracycline in acid but is stable to -ketone cleavage followed by lactonization to the isotetracycline in base.

• Reduction of the 6-hydroxyl group also dramatically changes the solubility properties of tetracyclines. This effect is reflected in significantly higher oil/water partition coefficients of the 6-deoxytetracyclines than of the tetracyclines (table below).

• The greater lipid solubility of the 6-deoxy compounds has important pharmacokinetic consequences. Hence, doxycycline and minocycline are absorbed more completely following oral administration, exhibit higher fractions of plasma protein binding, and have higher volumes of distribution and lower renal clearance rates than the corresponding 6-oxytetracyclines.
Derivatives have been synthesized with the 6-OH group been removed, these agents were more stable, lipophilic and long lasting than those with 6-OH group.
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<tr>
<th></th>
<th>Kₚc Octanol/Water pH 5.6</th>
<th>Absorbed Orally (%)</th>
<th>Excreted in Feces (%)</th>
<th>Excreted in Urine (%)</th>
<th>Protein Bound (%)</th>
<th>Distribution (% body weight)</th>
<th>Clearance (mL/min/1.73 m²)</th>
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<td>50</td>
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</table>


Neither 6α nor 6β-OH are essential for activity

1. Doxycycline is more active than oxytetracycline,
2. These derivatives are also more stable toward acid and base inactivation.
3. More lipid soluble ➔ better absorbed orally (>90% orally available).
4. High protein binding ➔ have long duration of action.

Methacycline is more active than oxytetracycline but unstable toward acid and base inactivation.

Methacycline

\[ t_{1/2} = 12 \text{ hrs} \]

more potent than 6-oxytetracycline
**C-7 and C-9 substituents:**
- The nature of the aromatic D-ring predisposes the C-7 position to electrophilic substitution.
- Substitution with electron withdrawing group such as nitro and halogen groups are introduced in some C-7 tetracyclines, which produces the most potent of all the tetracyclines *in vitro*, but some are potentially toxic and carcinogenic.
- The C-7 acetoxy, azido (N=N=N), and hydroxyl tetracyclines are weak in terms of antibacterial activity.
C-10 substituents:
The C-10 phenolic moiety is **necessary** for antibacterial activity.

C-11 substituents:
The C-11 carbonyl moiety is a part of one of the conjugated keto-enol system which is **necessary** for antibacterial activity. C-11a should not be modified.

C-12/12a substituents:
Esterification of OH leads increase lipophilicity and tissue distribution.
The ester should be remove to give active tetracycline.

OH group at 12a position is **necessary** for good antibacterial action.
• Most of tetracyclines are excreted unchanged in urine.
• Sulfate and glucurononide conjugates were detected in urine especially for Doxycycline and minocycline.
• The major metabolite found to be the N-dealkylated at C4, and to a little extent at C7 (for minocycline).

\[ N\text{-dealkylation By cytochrome P450 oxygenases in the liver} \]

\[ \text{Sulfate conjugation glucuronidation} \]
The active uptake of tetracyclines by bacterial cells is an energy-dependent process that requires adenosine triphosphate (ATP) and magnesium ions.

Mechanism of resistance include:
1. Lower penetration through porins
2. Efflux through transmembranal active transporters
3. Ribosomal protection by some cytoplasmic proteins
4. Enzymatic oxidation of tetracyclines
• Oxidation of tetracycline antibiotics by the enzyme, TetX (a flavin-dependent mono-oxygenases).

• TetX catalyzes the monohydroxylation of tetracycline antibiotics at position 11a, which disrupts the Mg2+ binding site of the antibiotic that is required for antibacterial activity.

• Subsequent to TetX-catalyzed hydroxylation, the antibiotic undergoes non-enzymatic rearrangement into unstable products that polymerize into a black product after several hours.

Fig. 13. Inactivation of oxytetracycline by TetX.
The differences between the antimicrobial spectra of various tetracyclines are not large.

They have the broadest spectrum of activity, on both gram +ve, gram –ve and atypical bacteria.

Resistance has been developed rapidly against tetracyclines, therefore, penicillins replaced them in many infections, especially the respiratory infections.

Tetracyclines are still used in rickettsia, Chlamydia, mycoplasma and acne infections.

Some of them have antiparasitic properties such as the use of Doxycycline in the treatment and prophylaxis of malaria.

They have bacteriostatic action, not recommended in life threatening infections such as septicemia, endocarditis and meningitis.
Stable chelate complexes are formed by the tetracycline with many metals, including calcium, magnesium and iron. Such chelates are usually insoluble in water.

The tetracyclines are distributed into the milk of lactating mothers and will cross the placental barrier into the fetus.

Their high affinity to calcium → deposition in bone and teeth.

Therefore, tetracyclines are not given to pregnant women or children.

• Phototoxicity.

Certain tetracyclines, most notably those with a C-7-chlorine (e.g. Chlortetracycline, Demeclocycline) absorb light in the visible region, leading to free radical generation and potentially causing severe erythema on exposure to strong sunlight.
Tetracycline is produced by
1. Fermentation of *Streptomyces aureofaciens* and related species
2. Or by catalytic reduction of chlortetracycline

It is a bright yellow, crystalline salt that is stable in air but darkens on exposure to strong sunlight. Tetracycline is stable in acid solutions with a pH above 2.

Tetracycline hydrochloride is also available in ointments for topical and ophthalmic administration. A topical solution is used for the management of acne vulgaris.
Tetracycline is 4-dimethyl amino-1,4,4a,5,5a,6,11,12a-octahydro-3,6,10,12,12a-pentahydroxy-6-methyl-1,11-dioxo-2-naphthacenecarboxamide
Chlortetracycline was isolated from *Streptomyces aureofaciens*.

It is similar to tetracycline, however has Cl on C7.

The hydrochloride salt is a crystalline powder with a bright yellow color, which suggested its brand name, *Aureomycin*.

Oral and parenteral uses are rare. It is used mainly as dermal and ophthalmic ointments.

Powder for veterinary medications.
• Demeclocycline is produced by altered strain of *Streptomyces aureofaciens*.

• It lacks the C6-methyl group of tetracycline, therefore, the C6-OH becomes secondary alcohol which is more stable than tetracycline against dehydration.

• It is most highly associated with phototoxicity due to C7-Cl.

• Produce dose-dependent diabetes insipidus (on long term of use).
• Isolated from *S. rimosus*.

• Oxytetracycline hydrochloride is a pale yellow, bitter, crystalline compound. The amphoteric base is only slightly soluble in water and slightly soluble in alcohol. It is odorless and stable in air but darkens on exposure to strong sunlight. The hydrochloride salt is a stable yellow powder that is more bitter than the free base.

• It is much more soluble in water, 1 g dissolving in 2 mL, and more soluble in alcohol than the free base. Both compounds are inactivated rapidly by alkali hydroxides and by acid solutions below pH 2. Both forms of oxytetracycline are absorbed rapidly and equally well from the digestive tract, so the only real advantage the free base offers over the hydrochloride salt is that it is less bitter. Oxytetracycline hydrochloride is also used for parenteral administration (intravenously and intramuscularly, mainly for veterinary uses).
Doxycycline (Cont.)

- Doxycycline is doxycycline, $\alpha$-6-deoxy-5-oxytetracycline
- Produced semisynthetically from tetracycline and oxytetracycline and methacycline molecules.
- Reduction of methacycline gives two epimers of doxycycline. The $6\alpha$-methyl epimer is more than 3 times as active as its $\beta$-epimer. Different orientation of methyl group slightly affects the shape of the molecule, and thus the activity.
- Absence of 6-OH results increases acid and base stability of doxycycline, improved absorption through GIT, as well as elongated the half-life which permits single-daily dosing.
Doxycycline

Doxycycline 6\(\alpha\)-methyl epimer (gray) and 6\(\beta\)-methyl epimer (yellow)

The change in direction of 6-methyl group slightly affects the general conformation of doxycycline, and consequently the antibacterial activity.

Epimerization \(\rightarrow\) change in conformation \(\rightarrow\) change in antibacterial activity
Minocycline is 7-dimethylamino-6-demethyl-6-deoxytetracycline.

It is most potent semi-synthetic tetracycline obtained by reductive methylation of 7-nitro-6-demethyl-6-deoxytetracycline.

It is acid and base stable as doxycycline, orally absorbed and has long half life.
Methacycline is 6-deoxy-6-demethyl-6-methylene-5-oxytetracycline.

- It is semisynthesized from oxytetracycline.
- It has similar spectrum to other tetracyclines.
- It is acid and base stable due to absence of 6-OH.
Rolitetracycline

• is a semi-synthetic tetracycline (pyrroldinylmethyltetacycline).

• Has broad-spectrum activity used especially for parenteral administration.

• Mainly used in rickettsia and brucellosis.

• Recent studies trying to investigate the effect of its combination with group of \( \beta \)-lactams such as cefotaxime for treating MRSA infections.
Glycyclcyclines & Tigecycline

- Is semisynthetic analogue of tetracycline developed to overcome bacterial resistance

- **Glycyclcyclines**
  - More active than old tetracyclines against the resistant strains.
  - Have broad spectrum of activity.
  - Has glycylamido side chain at C9

- **Tigecycline:**
  - Long $t_{1/2}$ of 55.4 hrs.
  - Highly bound to plasma protein.
  - Mainly used as IV for skin infections and intra-abdominal infections.
  - Tigecycline is not affected by the two major tetracycline resistance mechanisms, ribosomal protection and efflux. Accordingly, tigecycline has activity vs broad spectrum of bacterial pathogens.
  - Has N,N-dimethyglycylamido at C9