Aya AL-majali

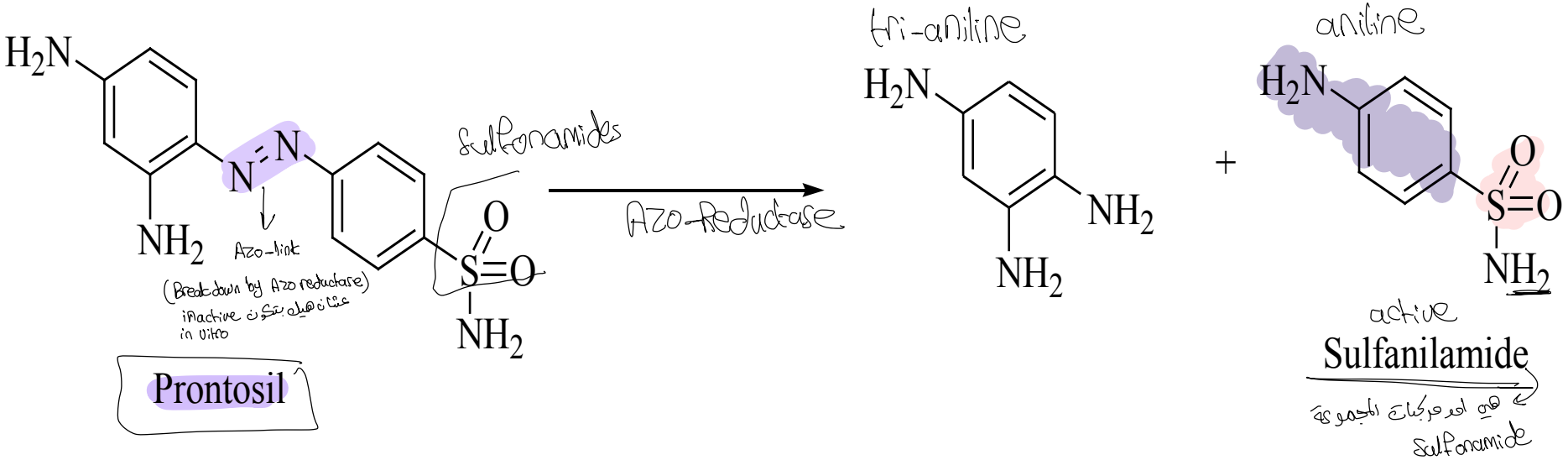
Sulfonamides antibacterial agents (The first synthetic antibacterial agents)

Dr. Rand Omar Shaheen

Hashemite University

Sulfonamides antibacterial agents

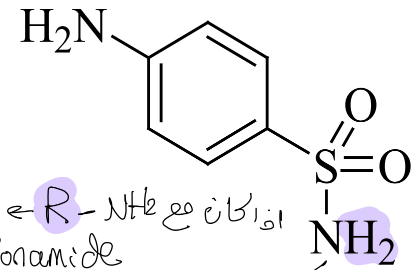
- In 1932. Domagk began to study a brilliant red dye called prontosil. This dye showed *in-vivo* antibacterial activity while it was *in-vitro* inactive (Pronsasil is inactive on bacterial culture).
- Later it was found that prontosil has to be activated by the in vivo metabolic pathways to give the active form.



(*) والسبب انو هاي الصيغة هي active في vivo انو هاي البكتريا تفرز Azo reductase داخل جسم الانسان بتكسر هاي الرابطة
(*) Sulfasalazine تحتاج هذا الانزيم لحتى تصبح فعالة

Nomenclature of Sulfonamides (Cont.)

- I. Antibacterials that are aniline-subsitiuited . Sulfonamides (the Sulfanilamides)



اذا كان مع NH_2 – R ← منسميها
Sulfonamide ← المجموعة الاولى

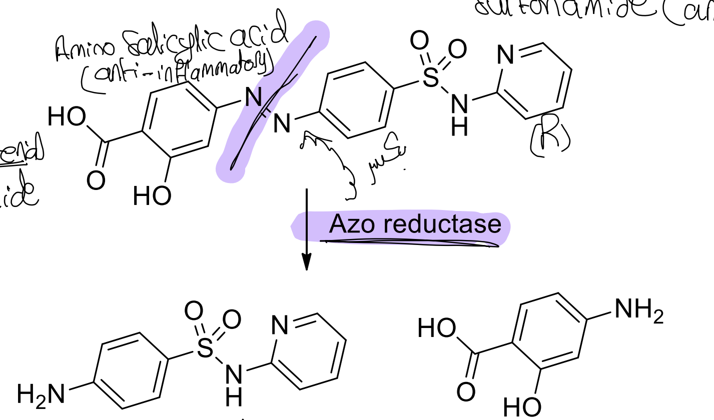
Sulfanilamide
not Sulfonamide

- II. Prodrugs that react to generate active Sulfanilamides (i.e Sulfasalazine)

بعالج ال chronic bowel disease
مجموعة أمراض تخلي جسم المناعة يهاجم الجهاز الهضمي ويعمل تقرحات فيها

Sulfasalazine → anti-inflammatory salicylic acid + antibacterial sulfonamide

Sulfonamide (anti bacterial)
Amino Salicylic acid (anti-inflammatory)



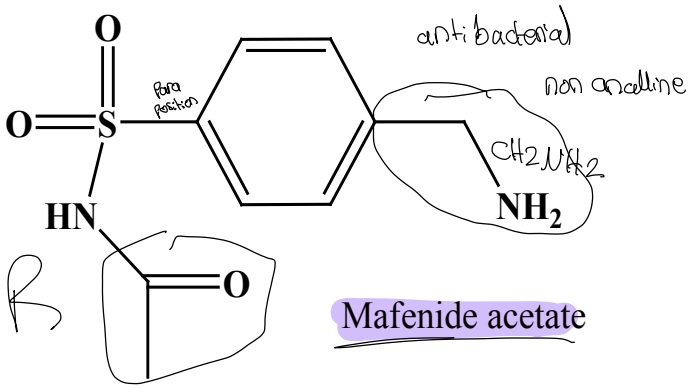
Sulfapyridine

5-amino salicylic acid

(*) first line to treat IBD → ciprofloxicine
(*) second line IBD → Sulfonamide
irritable bowel Disease

Nomenclature of Sulfonamides

- III. Nonanaline sulfonamides (i.e., mafenide acetate)

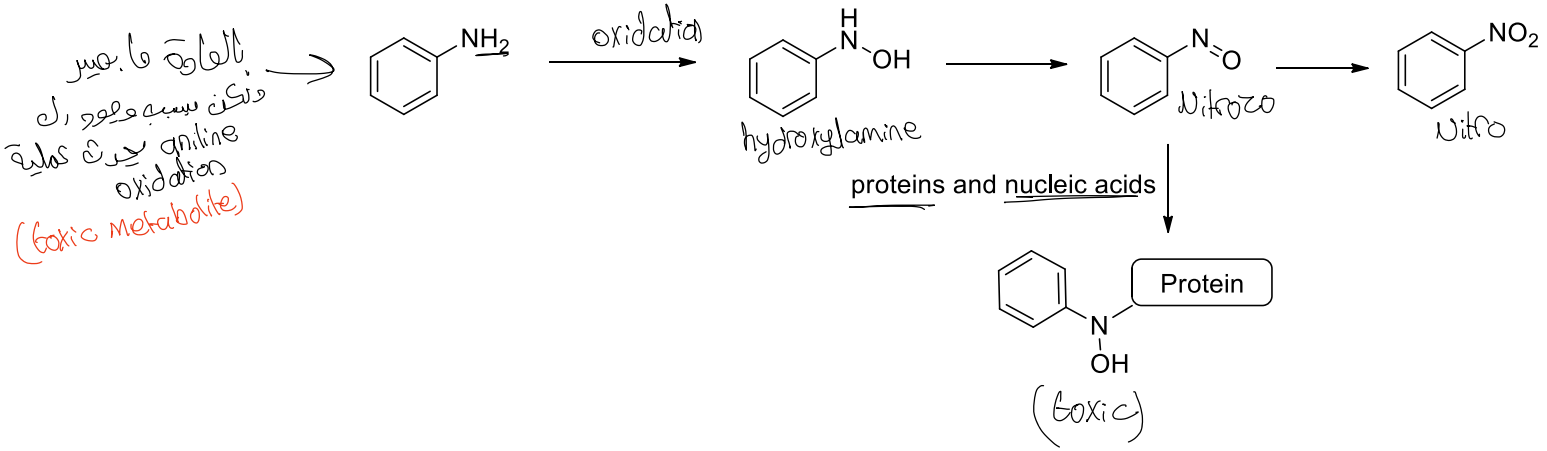


Mafenide acetate

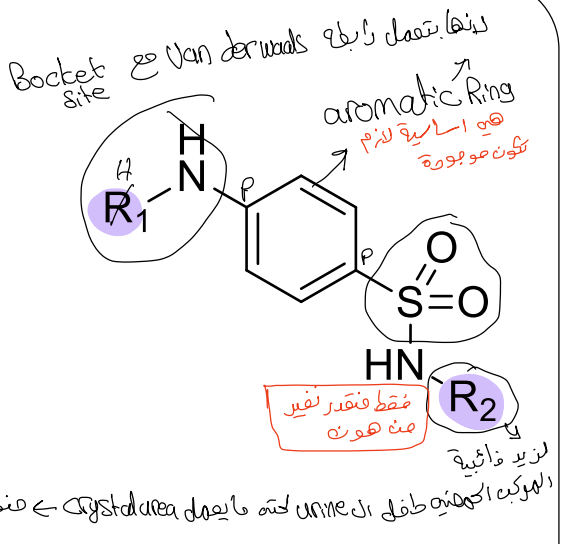
Sulfonamides antibacterial agents

- Their bacterial activity is mainly on gram +ve and -ve bacteria
- limitation of the sulfa drugs use:

1. Sulfa allergic reactions.
2. The formation of crystalluria. ← فيان S.A (Sulfonamide) في البول قليل ذرع تعمل مشاكل في الكلى
3. They give toxic metabolites after the oxidation of the aromatic amine:



SAR of sulfonamides



1. *P*-amino group is essential for activity and should be free (unsubstituted)
   - The sulfonamide nitrogen must have an attached hydrogen with a pKa similar to that of PABA (~6.5).
2. In the case of prodrugs (Sulfasalazine) the azo linkage that will be hydrolyzed to give the active free form.
3. The aromatic ring and the sulfonamide group are important for activity.
4. The sulfonamide and the amino group must be directly attached to the ring and in *P* position to each other.
5. Any extra substitution will reduce activity.
6. Sulfonamide nitrogen must be either primary (R-NH2) or secondary (R-NHR).

مهم لتصنيع القواعد النيتروجينية وال DNA
folic acid is methyl donor

SA يقتل خلايا البكتيريا ولا يؤثر على خلايا الانسان لانه الانسان لا يتأثر بنقصان انزيم Dihydropteroate synthetase كونه يتناول المنتج النهائي الي هو folic من الطعام على عكس البكتيريا رح يتأثر وتقل كمية folic acid الذي يعتبر Methyl donor مهم لتصنيع DNA الخلايا

Mechanism of action

- Sulfonamides are a competitive reversible inhibitors of **dihydropteroate synthetase** (DHP) which is a vital enzyme for the synthesis of tetrahydrofolate (Coenzyme F). Tetrahydrofolate is important for pyrimidine nucleic acid synthesis so the bacteria can no longer grow and divide which gives time for the host immune system to destroy the bacterial cells.

موجود بكثرة في البكتيريا وغير لازم للانسان (مهم لتصنيع folic acid)

لانه بأثر على انزيم

- Because of that sulfonamides have **bacteriostatic** effect not bactericidal so is not recommended in patients with weak or impaired immune system

لانه statics نفتقر على weak immune system

شخص AIDs مثلاً وكمان نزل كلية ← ما بعطيهم sulfonamide لازم اعطيهم Penecilline لانه bactericidal

Mechanism of action

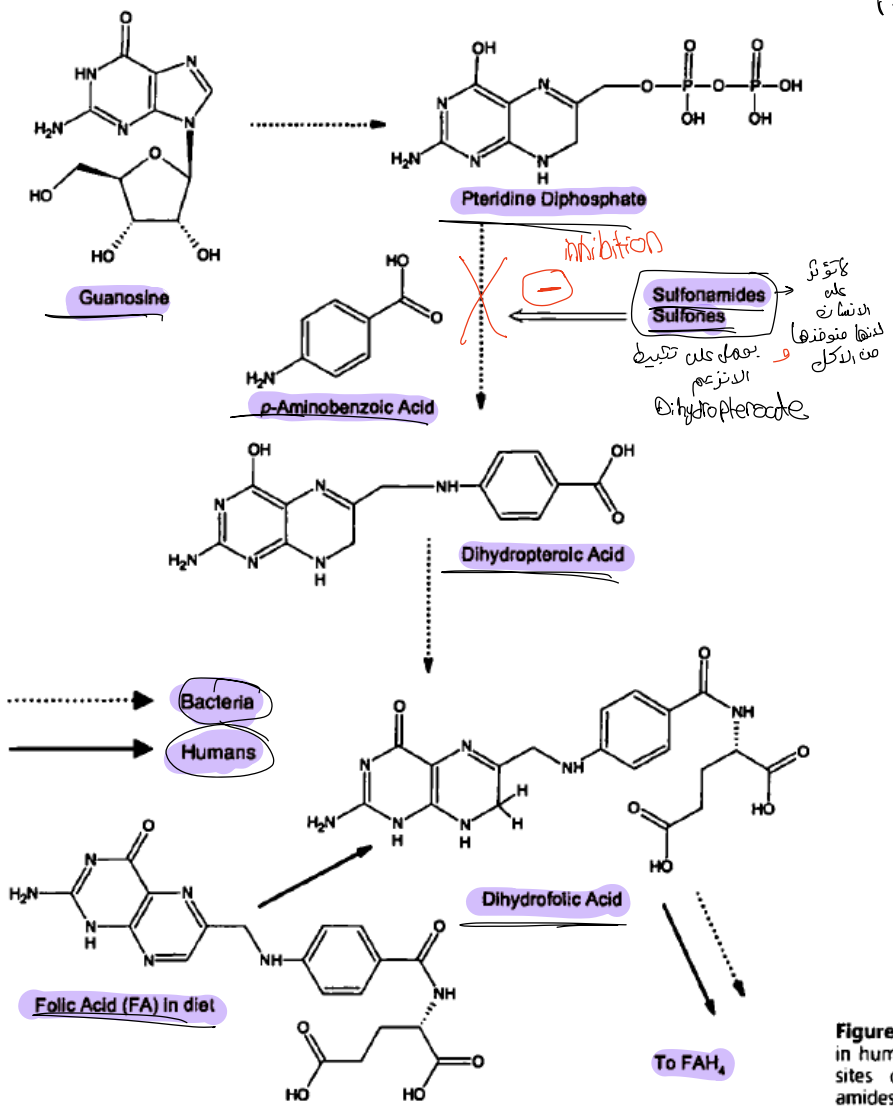


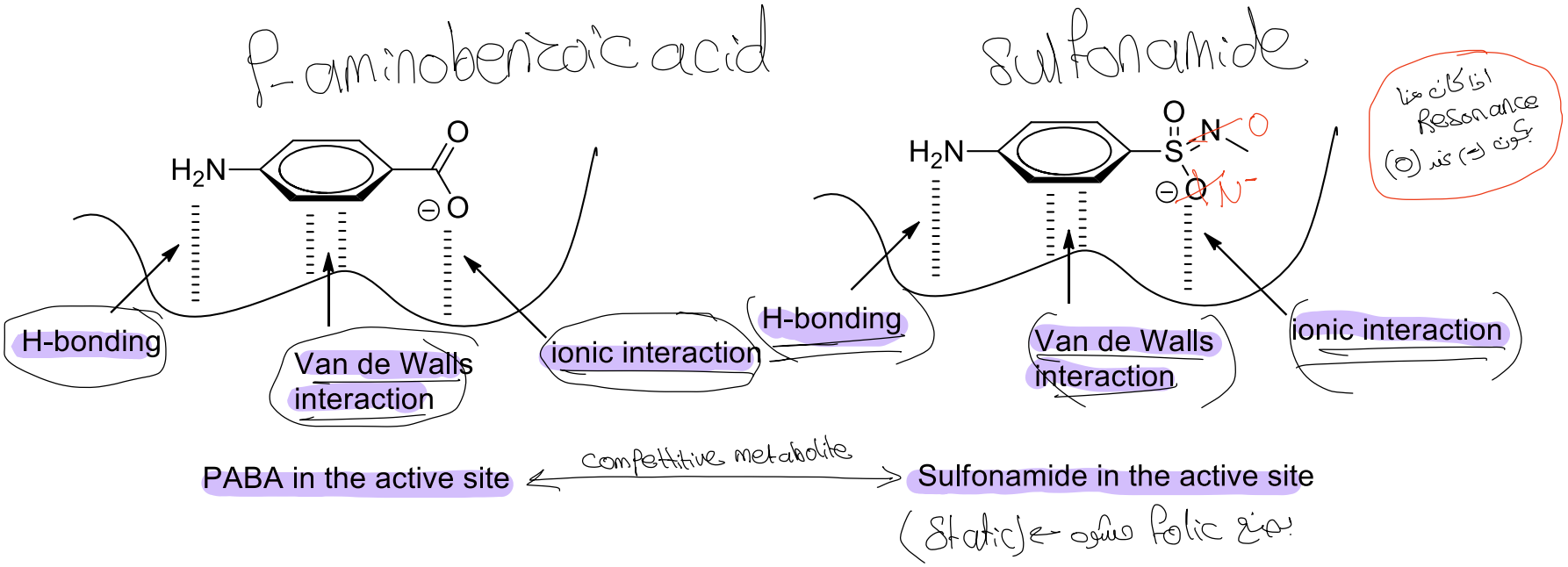
Figure 8–10 ■ Folate pathw in humans and bacteria and sites of inhibition by sulf amides and trimethoprim.

Referance: Wilson and Gisvold's Textbook of Organic

Mechanism of action

- Sulfonamides mimic *P*-aminobenzoic acid (PABA) which is the normal substrate for dihydropteroate synthetase. This means that sulfonamide will bind in the same manner as PABA:

يعني اللي تركيزه اعلى هو اللي يوتبط بال انزيم



Mechanism of action

- Because sulfonamides are reversible competitive inhibitors for the enzyme, the bacteria can increase the production of PABA to compete with sulfonamide at the active site and become resistant to sulfa drugs.
- In such case, the dose of sulfonamide agents should be increased to overcome this resistant mechanism. But this high dose is accompanied with an increase in side effects especially the crystalluria.
- N4 acetylation reduces drug solubility, which may result in precipitation in the urine leading to crystalluria. Increasing the pH of urine with a systemic alkalizer along with increased water intake will decrease the risk of this potential adverse effect.

Mechanism of action

- In human, the cell synthesized tetrahydrofolate from folic acid that obtained from food sources. This folic acid is normally transported to inside the cell by special transport system.
- Bacterial cell does not have such transport system and they should synthesize tetrahydrofolate using PABA.
- For that reason, human cells do not need dihydropteroate synthetase enzyme which means sulfonamides have selective antibacterial activity.

Mammalian Folate biosynthesis

* Dihydrofolate Reductase
يتم تثبيطه
- human: methotrexate
- Bacterial: trimethoprim
- Protozoal: Pyrimethamine

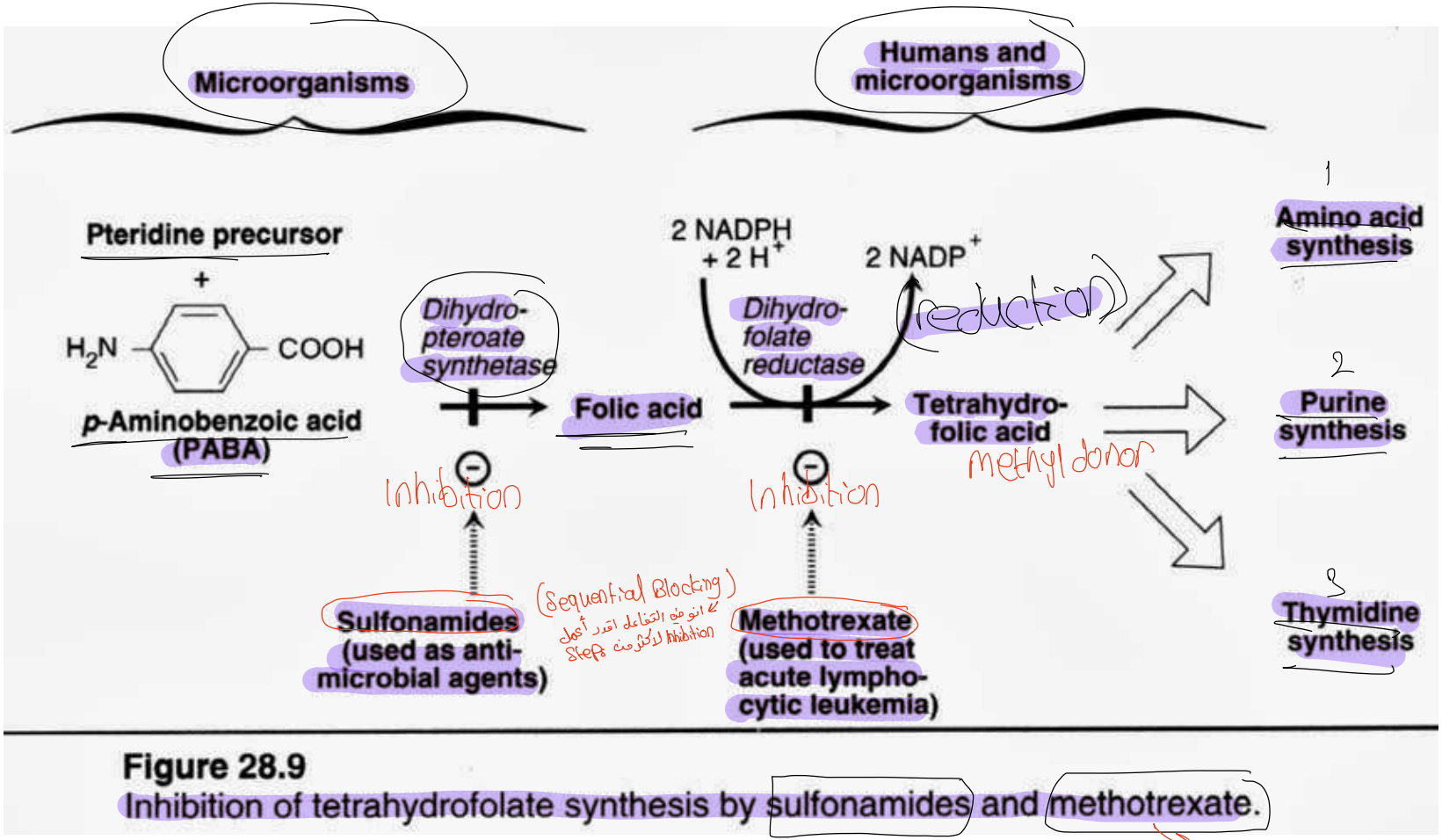
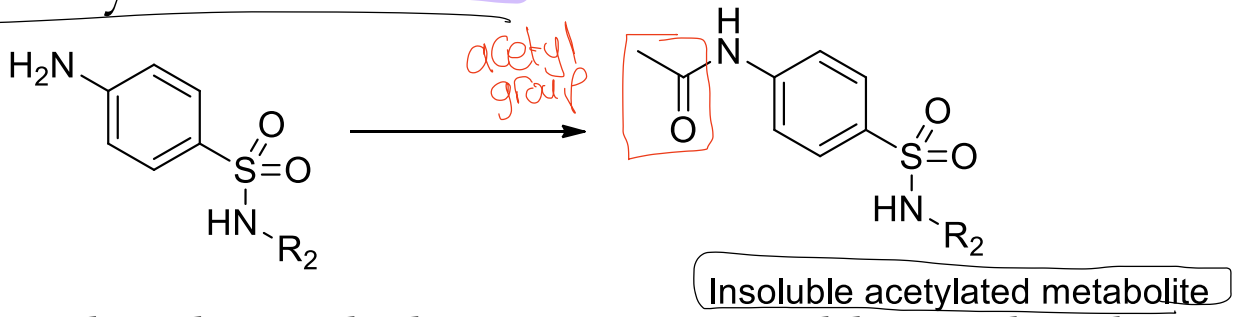


Figure 28.9
Inhibition of tetrahydrofolate synthesis by sulfonamides and methotrexate.

human

The problem of crystalluria

- Sulfonamides are mostly excreted in urine as acetylated metabolite.
- They are relatively water insoluble mainly due to the formation of the acetylated metabolites.



- The acetylated metabolite is non-ionizable under the pH conditions of the urine (≈ 7) that increase the possibility of precipitation and the formation of crystals in the urine (crystalluria)

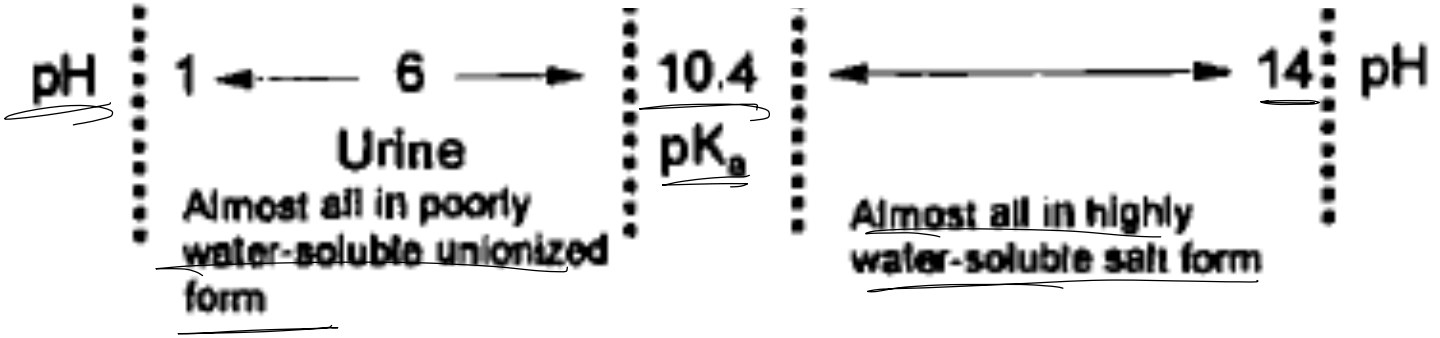
The problem of crystalluria

- How to minimize the possibility of crystalluria formation with sulfonamides:
  - Increase the urine flow.
  - Increase the pH of the urine to increase the ionization of sulfonamides and the formation of water soluble salts (this can be done by taking sodium bicarbonate or potassium citrate.
  - Lowering the pKa of the sulfonamide group which will help to increase the ionization under the acidic conditions. This can be done by adding electron withdrawing group on the sulfonamide side chain

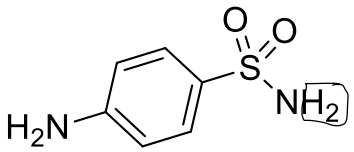
TABLE 8–8 pK_a Values for Clinically Useful Sulfonamides

Sulfonamide	pK_a
Sulfadiazine	6.5
Sulfamerazine	7.1
Sulfamethazine	7.4
Sulfisoxazole	5.0
Sulfamethoxazole	6.1

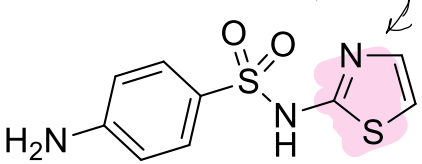
pka د تلوب



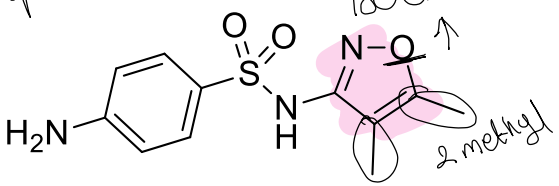
Sulfonamides with reduced crystalluria formation



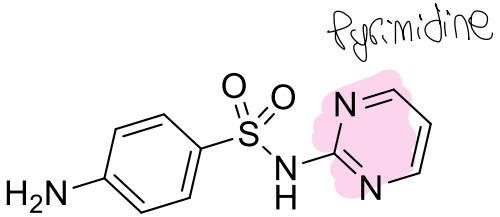
Sulfanilamide pKa = 10.4



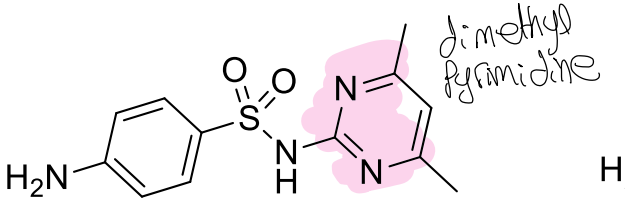
Sulfathiazole pKa = 8.5



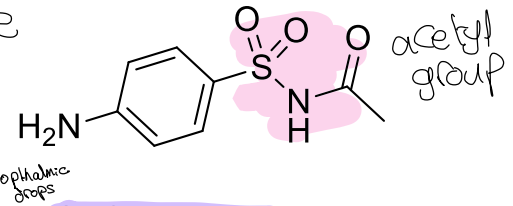
Sulfisoxazole pKa = 5.0



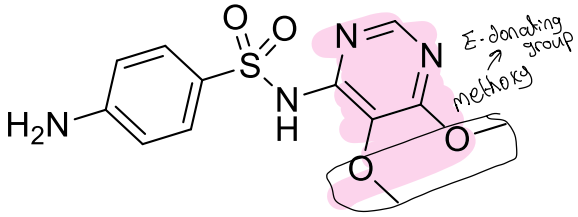
Sulfadiazine pKa = 6.5



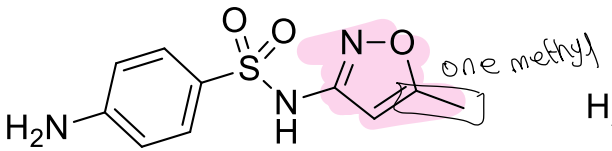
Sulfamethazine pKa = 7.4



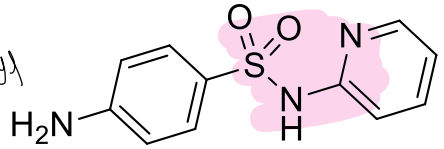
Sulfacetamide pKa = 5.4



Sulfadoxine pKa = 8.1



Sulfamethoxazole pKa = 6.1



Sulfapyridine pKa = 8.4

Clinical Application

The drugs are effective against both gram-positive and gram-negative organisms, but bacterial resistance and newer more effective drugs have replaced the majority of the previously available sulfonamides.

Today, many of the sulfonamides have been discontinued, but some are still available and are primarily limited to treatment of susceptible gram-negative organisms.

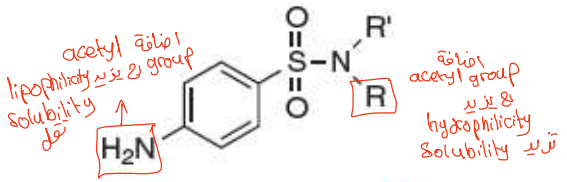
Products containing sulfonamides are shown in Table 23.1.

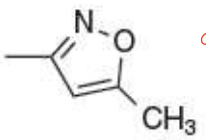
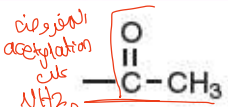
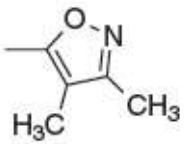
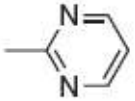
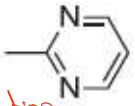
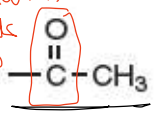
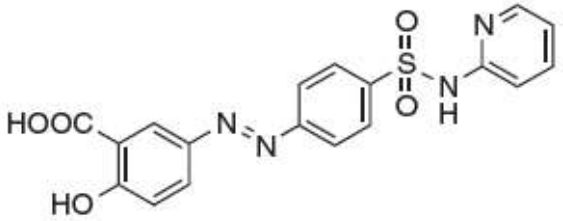
Sulfisoxazole, in the form of the prodrug N1-acetylsulfisoxazole, is used in combination with erythromycin ethylsuccinate (EES) and indicated for the treatment of **otitis media.**

1. Sulfamethoxazole in combination with trimethoprim (see below) is used to treat **uncomplicated urinary tract infections,**
2. while sulfadiazine when combined with the antiprotozoal agent pyrimethamine is used to treat ***Toxoplasma gondii* infections.**
3. Silver sulfadiazine is used topically to treat burns, with both the sulfa drug and the silver ion having antibacterial activity.
4. Sodium sulfacetamide is a water-soluble preparation used to treat ophthalmic
5. infections, while sulfasalazine is effective in the treatment of **ulcerative colitis.** It is only poorly absorbed from the GI tract where it is hydrolyzed by intestinal

Clinically relevant sulfonamides

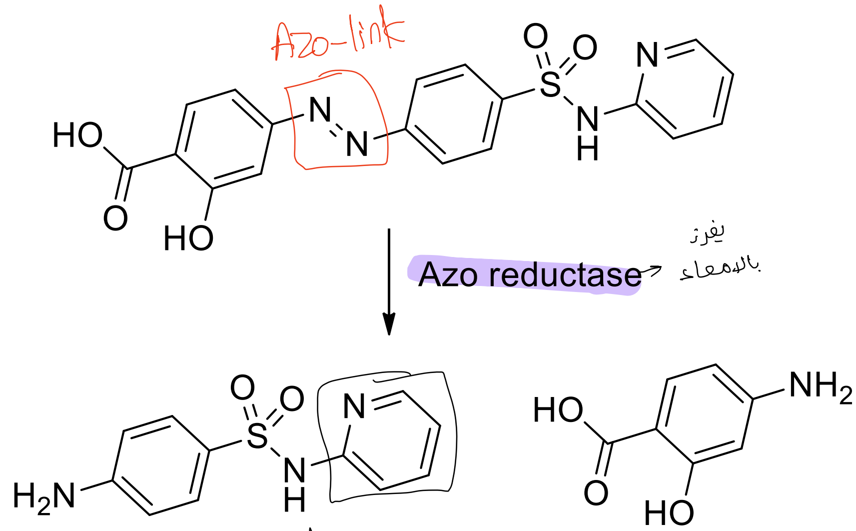
Clinically Relevant Sulfonamides



Drug: Generic Name	Product	R	R′	pKa
Sulfisoxazole acetyl (prodrug)	In combination with erythromycin ethylsuccinate			5.6 after hydrolysis
Sulfamethoxazole	In combination with trimethoprim		—H	5.0
Sulfadiazine	Oral dosage form		—H	6.52
Silver sulfadiazine	Topical dosage form			
Sulfacetamide sodium	Ophthalmic dosage form			5.4 free acid
Sulfasalazine	Gastrointestinal oral dosage form			

Sulfonamide prodrugs

- Sulfasalazine:
  - Used in local intestinal infections.
  - Gives sulfapyridine and 5-aminosalicylic acid upon the breakdown of the azo bond.
  - Used mainly in ulcerative colitis.



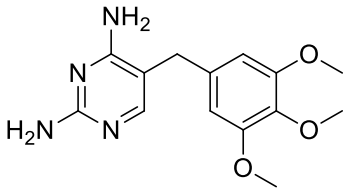
Sulfapyridine

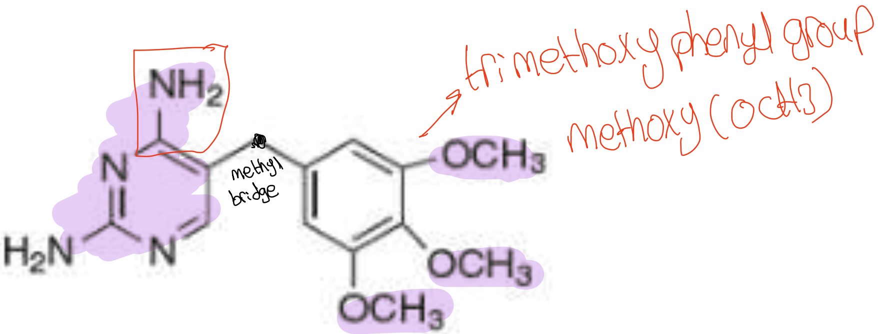
5-amino salicylic acid

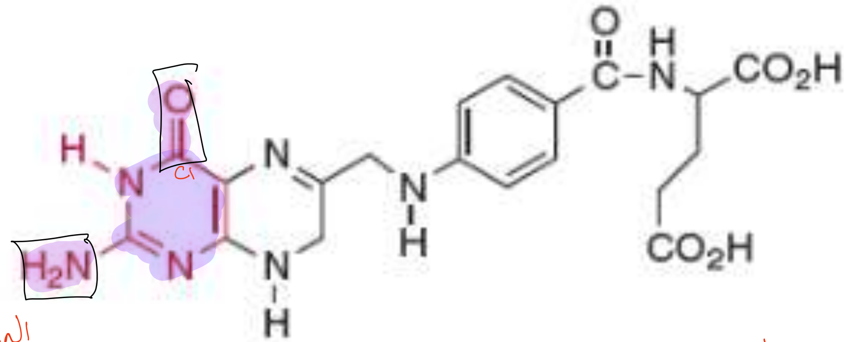
على الرغم من انو pKa=8.4 عالية ومثل ما درسنا لازم اعدل عليه لحتى اتخلص من (crystal urea)
ممكن هذا الدواء انا بدي اياه يكون هيك وما اله داعي نعدل له
امتصاص في المعدة و الامعاء

Other folate reductase inhibitors

- Trimethoprim:
  - Inhibits dihydrofolate reductase: this enzyme has human homologue but they do not have that much similarity in structure…. Therefore trimethoprim is 1000 more active on the bacterial copy of this enzyme..
  - Normally used in combination with sulfamethoxazole (cotrimoxazole):
    1. Lower dose from both drugs means less side effects.
    2. More effective than the monotherapy since they are targeting two different enzymes in the same metabolic pathway… this is what is called sequential blocking.







Trimethoprim (Proloprim, Trimpex)
Trimethoprim + sulfisoxazole (Co-Trimoxazole)
Trimethoprim + sulfamethoxazole (Bactrim, Septra)

Dihydrofolic acid

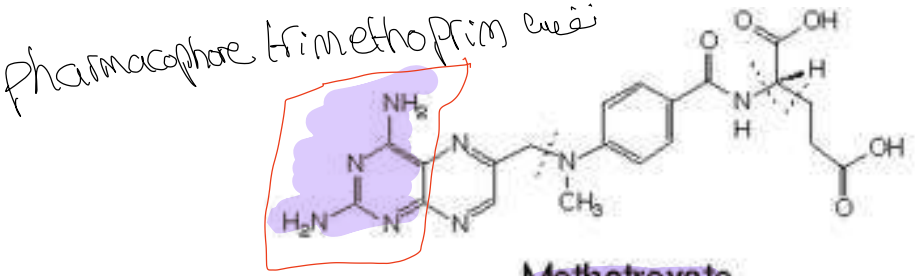
المفروض (trimethoprim + sulfamethoxazole)
هو الاسم التجاري غالب على الاسم العلمي (معروف اكثر)

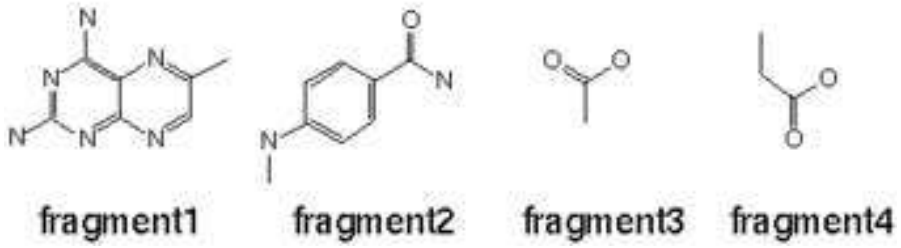
Is natural substrate of dihydrofolate Reductase

(اللي باللون الاحمر بالشكل)

* all Dihydrofolic acid + trimethoprim + methotrexate have the same pharmacophore units is Pyrimidine + NH2, but different in C1 → Dihydrofolic → C1 is carbonyl & trimethoprim → C1 is NH2, methotrexate → C1 is NH2
(فقط يربط بـ Binding pocket تبعت الـ Reductase enzyme

- There is another dihydrofolate reductase
- inhibitor –for Human enzymes (**Methotrexate**)
- which is used as anticancer drug .
- There is another one too, (**pyrimethamine**), as a malarial dihydrofolate reductase inhibitor





as combination

- Sulfonamide + Trimethoprim are :

(1) • Septra®

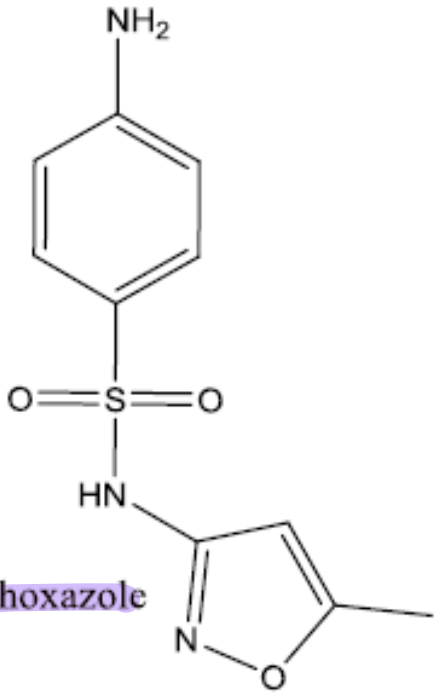
(2) • Balkatrin® . great gram(-)/gram(+) bacteria

- Another use of Sulfadrug is for Protozoa (eukaryotic cells) like Amoeba & Malaria, and the most important use is to treat **Pneumocystis carinii** (Pneumonia) in AIDS patients, (Sulfamethoxazole + Trimethoprim).

Combination is Static action

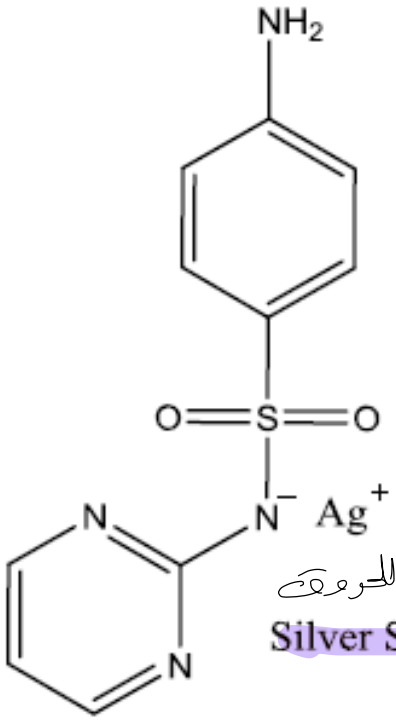
*Sulfadugs are divided to : Short(Sulfacetamide)(1), Moderate(2), Long acting(3).

UP



Sulfamethoxazole

DOWN



Topical للحروق

Silver Sulfadiazine

Protein binding of sulfonamides

- Vary in plasma protein binding: Sulfaisoxazole… ~~76~~ 37%, Sulfamethoxazole… 60%, sulfadiazine…. 38%.
- The fraction that is protein bound is not available for enzyme inhibition, therefore this fraction is inactive.
- The protein binding is a reversible process, so there will be a gradual release of sulfonamide which will become available.
- Factors affecting protein binding of sulfonamides:
  1. Lipophilicity of the structure.
  2. Substitution on the free amine will increase protein binding (such as the acetylayed metabolite is more protein bound than the parent sulfonamide).

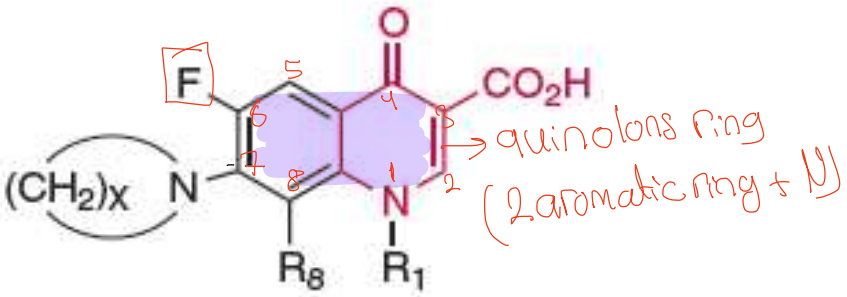
Use :

- The original Sulfanilamide was used against most of the infections, Upper and Lower respiratory tract infections (Pneumonia mainly), it was the only treatment available.
- Their use was mainly for UTI (because it's eliminated quickly & their spectrum covers G-ve bacteria), it's still used until now
- **Spectrum**
- Broad spectrum (G+ve & G-ve) bacteria , with time, development of resistant happens

Protein binding of sulfonamides

- Since albumin is basic, acidic and neutral drugs will primarily bind to albumin.
- If albumin becomes saturated, then these drugs will bind to lipoprotein.
- Basic drugs will bind to the acidic alpha-1 acid glycoprotein.
- Protein binding can influence the drug's biological half-life in the body but this relationship still not clear since some drugs with low protein binding have long duration of action (sulfisoxazole: protein binding 37% and half life is 17 hours).

(2) 4-Quinolones (Synthetics) not antibiotics



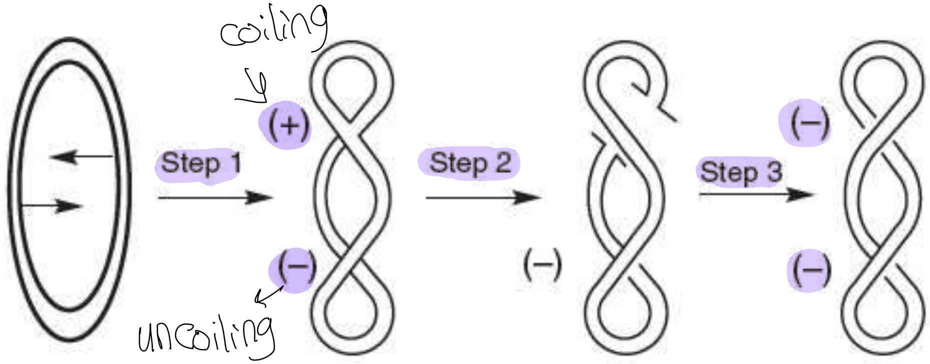
4-Quinolone
(pharmacophore shown in red)

The fluoroquinolones have been found to be effective in treatment of various bacterial infections (urinary tract infection) depending on the nature of the substitution on the 4-quinolone pharmacophore.

MOA

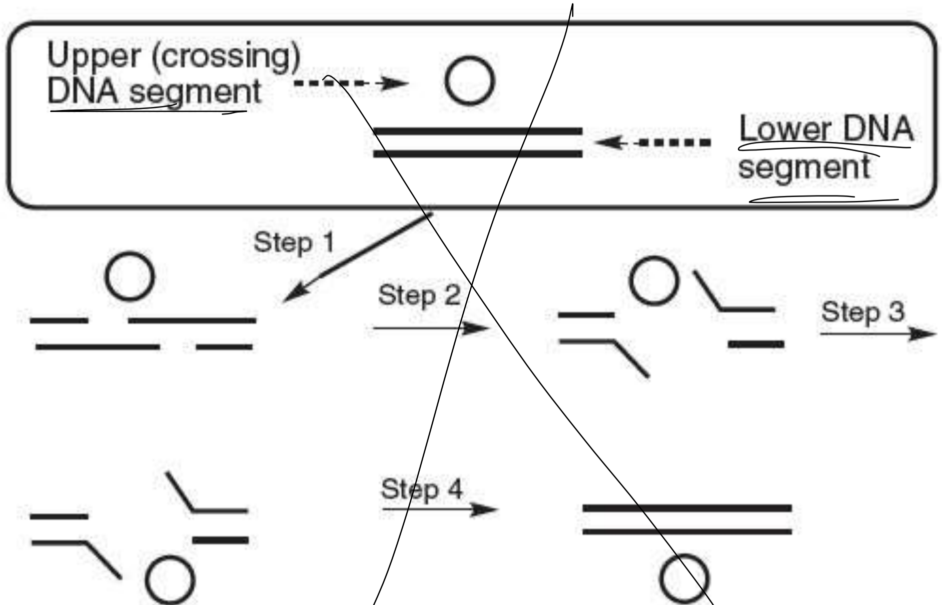
- Irreversible inhibitors of DNA gyrase and topoisomerase IV, key enzymes involved in DNA-dependent RNA polymerase (DDRP). DNA dependant RNA polymerase → (DDRP)

* اذا منعت تصنيع DNA يصير ما يتم protein
Bacteriocidal
Not Static
Fluoroquinolones في الدماء (DDRP enzyme) على ما أطلق عليه انزيمين هما (gyrase & topoisomerase hormone)
RNA هو الي يصنع نسخ
الانزيم المصنع لـ DNA و RNA
* ماكنت هوني انزيم RNA المعتمد على DNA



A. View from the top: Step 1. Stabilize positive node. Step 2. Break both strands of the back segment. Step 3. Pass unbroken segment through the break and reseal on the front side.

الية العمل
gyrase +
topoisomerase



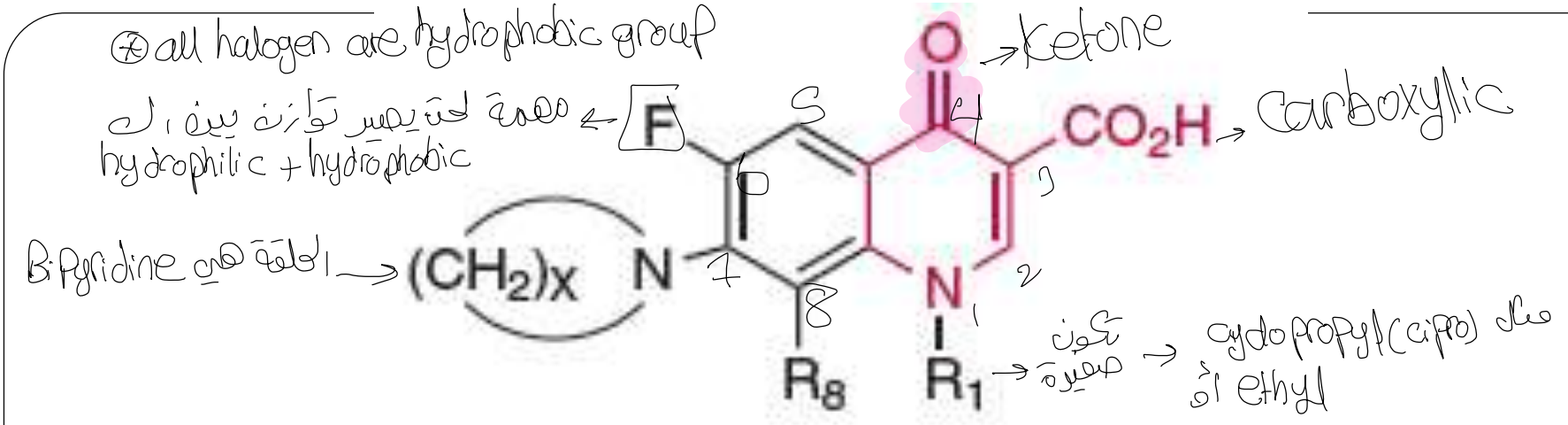
B. View from the side: Step 1. Staggered cuts in each strand. Step 2. Gate opens. Step 3. Transverse segment passed through the break. Step 4. Reseal cut segment.

* DNA الي بجسم الانسان هي سلسلة طويلة كله شكل Supercoiled (مفتولة)
ولحتى يشتغل RNA Polymerase لازم تبطل مشدودة والانزيمين (topoisomerase + gyrase) هما الي بفتحوا الفتلة تبعت DNA

* الفتلة عبارة عن (−) و(+) يعني وصلة امام وثانية خلف اما اذا كانت الفتلة (−) و(−) أو (+) و(+) اح تفك الفتلة

* الية عمل الانزيم (topoisomerase+gyrase) :-
(1) Stabilization of DNA
(2) Break both stand of back segment + Pass unbroken segment throug the break and reseal on the front side

يعني بقطعها وبقدمها للامام وبلزقها رح تتحول الفتلة (−) و(−) وهيك رح يشتغل الانزيم



SAR

- The quinolone pharmacophore is essential for activity through binding to the DNA gyrase (Table 23.2).
- R_1 is important for potency and commonly consists of an ethyl or cyclopropyl.
- **Fluoro at C6** improves penetration of the bacterial cell wall through improved hydrophobicity.
- Heterocyclic substitution at C7 affects the spectrum of activity against gram-negative bacteria.
- R_8 affects spectrum of activity as does R_1/R_8 linked forming a third ring in the molecule (finafloxacin).

Physicochemical and Pharmacokinetic Properties

- • 4-Quinolones are incompatible with heavy metals (e.g., Ca^{2+}, Mg^{2+}, Zn^{2+}, Fe^{2+}, Al^{3+}) due to an insoluble chelate resulting from bonding between the metal and the C3 carboxyl and C4 ketone.
- • 4-Quinolones may cause skin phototoxicity upon exposure to the sun (UV A radiation)

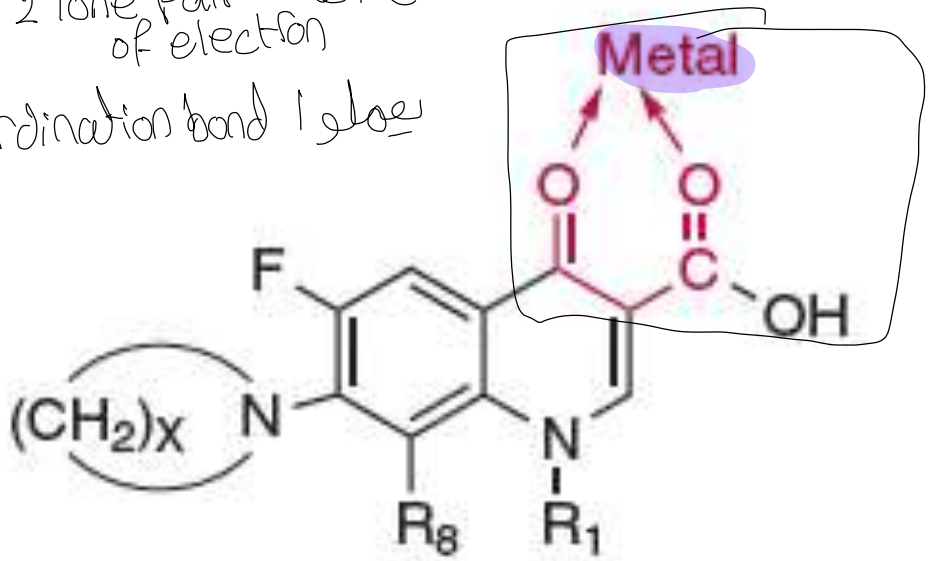
ADVERSE EFFECTS

4-Quinolones: GI disturbance: nausea, vomiting, and abdominal discomfort. CNS effects: headache and dizziness, but may also include hallucinations, insomnia, and visual disturbances due to binding of lipophilic drugs to GABA receptors. Several analogs caused QT prolongation leading to their removal from the market.

⊛ غالبًا انزيمات اللي بتتسبب قطع مثل gyrase بتكون (metaloenzyme) يعني تحتوي على Metal في تركيبها الكيميائي
وال Metal تعمل coordination bond وهي رابطة قوية جدًا يصعب فكها

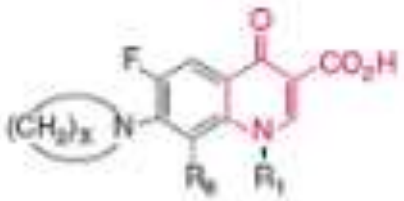
Binding of the drug to DNA gyrase involves the carboxyl and the ketone.

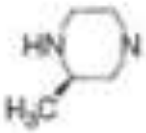
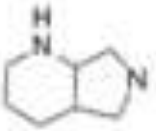
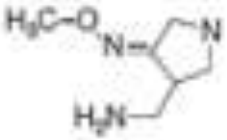
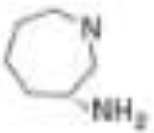
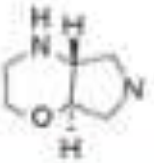
⊛ MoA of metaboenzyme of gyrase:
1) Metal عنده 2 lone pair of electron
2) carbonyl group (C3+C4)
coordination bond يعني



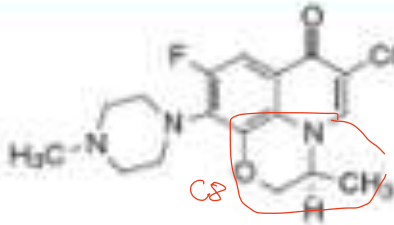
Metal chelation

مطلوب فقط الي فوق والباقي
لتعرف التنوع الي على Structure



Drug: Generic Name	Trade Name	R_1		R_8
Norfloxacin	Noroxin	C_2H_5		H
Ciprofloxacin	Cipro			H
Gatifloxacin	Tequin	△		CH_3O
Moxifloxacin	Avelox	△		CH_3O
Gemifloxacin	Factive	△		
Besifloxacin	Besivance	△		Cl
Finafloxacin*	Xtoro	△		N≡C

cyclopropyl



Ofloxacin (Racemic)(Floxin)
Levofloxacin (1-S)(Levaquin)

Clinical Application

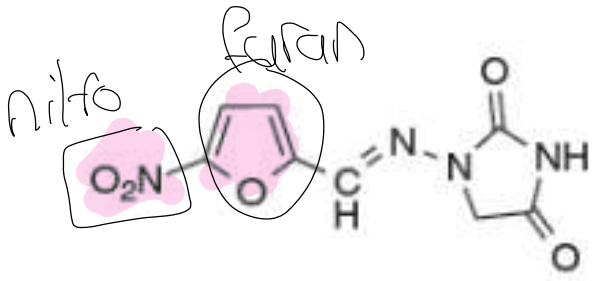
- The fluoroquinolones represent a potent class of **bactericidal** agents with utility in a variety of infectious conditions. **The most common** indications include **UTIs** caused by sensitive organisms; prostatitis; some sexually transmitted infections; respiratory infections; and bone, joint, and soft tissue infections.

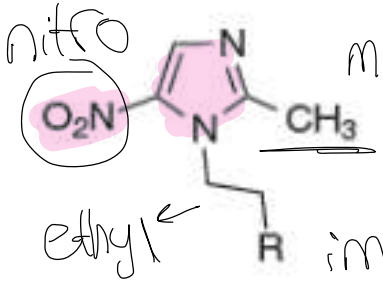
bone + joint + soft tissue
هدف تركيزهم قليلة
يعني ما بوصلهم الدواء
ف انا بحاجة لدواء يكون
له volume of Destribution
عالي

Synthetic

Nitroheteroaromatic Compounds

(3) *Nitrofurantoin* furan (O + حلقة) + nitro





imidazole + nitro group

Nitrofurantoin
(Furadantin, Macrodantin)

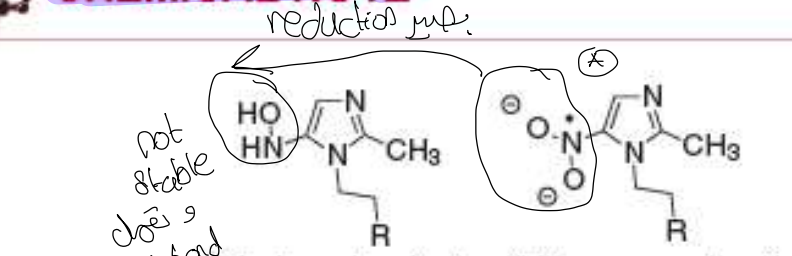
Metronidazole, R = OH (Flagyl) anaerobic gram-ve Bacteria
Tinidazole R = $SO_2C_2H_5$ (Fasigyn)
ethyl sulfoxide

- Nitrofurantoin is the only nitrofuran which remains available and is used for treatment of uncomplicated UTIs.

Metronidazole

- Metronidazole and tinidazole are used to treat some bacterial infections (e.g., GI tract peptic ulcer, pseudomembranous colitis) and protozoal infections (e.g., giardiasis, trichomoniasis).

CHEMICAL NOTE

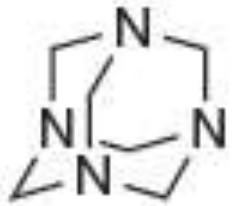


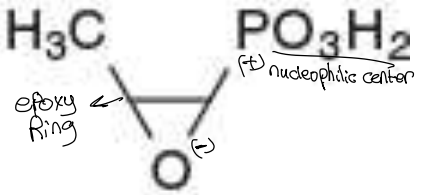
Possible active form of nitro aryls

MOA

- Most likely, the nitroheteroaromatic compounds are prodrugs in which **the nitro group is reduced** to the active hydroxylamine or nitrogen radical which interferes with DNA and or RNA.

Methenamine and Phosphomycin





Methenamine
(Prosed, Urimax, Urised, Uroqid-Acid)

Phosphomycin
(Monurol)

limited values and are used in uncomplicated UTIs.

MOA

- Methenamine is a prodrug, which in acidic urine generates ammonia and formaldehyde. The latter forms a Schiff's base with bacterial protein resulting in antibacterial action.
- Phosphomycin, through alkylation of a key sulfhydryl group in a bacterial transferase essential in cell wall glycoprotein synthesis; inhibits bacterial growth.