Chapter 2: β-Lactam Antibiotics

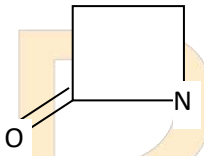
Syllabus:

Historical background, Nomenclature, Stereochemistry, Structural Activity relationship, Chemical degradation, classification and important products of the following classes

β-Lactam antibiotics: Penicillin, Cepholosporins, β- Lactamase inhibitors, Monobactams

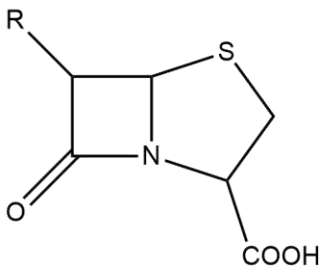
2.1. B-LACTAM ANTIBIOTICS

- β-lactam antibiotics consists of a β-lactam ring (4-Membered cyclic amide).
- Penicillins, Cephalosporins, Carbapenems*, Monobactams* (*Beta-lactamase resistance)

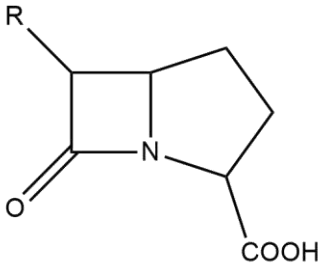


- β-lactam antibiotics are the most important class, that frequently used in the treatment of bacterial infection, these are the bactericidal drugs means they kill the bacteria by inhibiting cell wall synthesis.
- β-lactam antibiotics can further classify based on their structural basis (ring fusion with β-lactam ring).

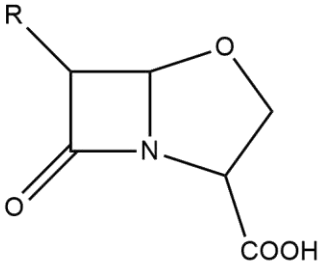
A) Saturated five-membered Ring: Penams (thiazolidine ring fused with β-lactam; Penicillin), Carbepenams (pyrrolidine ring fused with β-lactam), & Oxipenams (Oxazolidine ring fused with β-lactam)



Penam

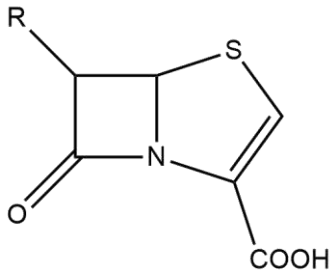


Carbapenam

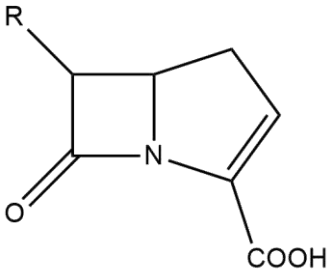


Oxapenam

B) Unsaturated five-membered Ring: Penems (dihydrothiazole ring fused with β-lactam), Carbepenams (dihydro-1H-pyrrrole ring fused with β-lactam)

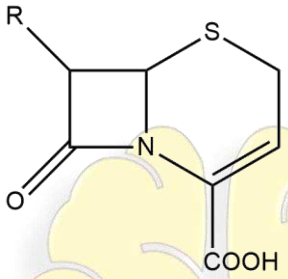


Penem

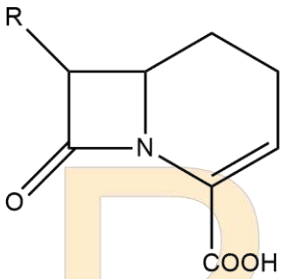


Carbapenem

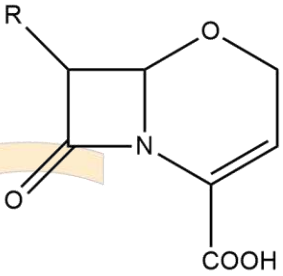
C) Unsaturated Six-membered Ring: Cephems (dihydro 1,3 thiazene ring fused with β-lactam; Cephelosporins), Carbecephem, Oxacephems



Cephem

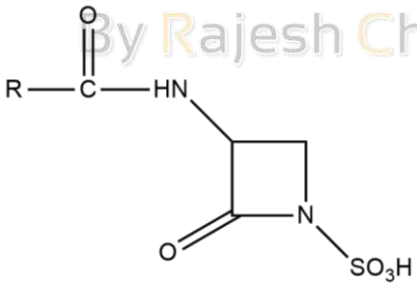


Carbacephem



Oxacephem

D) B-Lactam not fused with other: Monobactam (Aztreonam and Tigemonam)



Monobactams

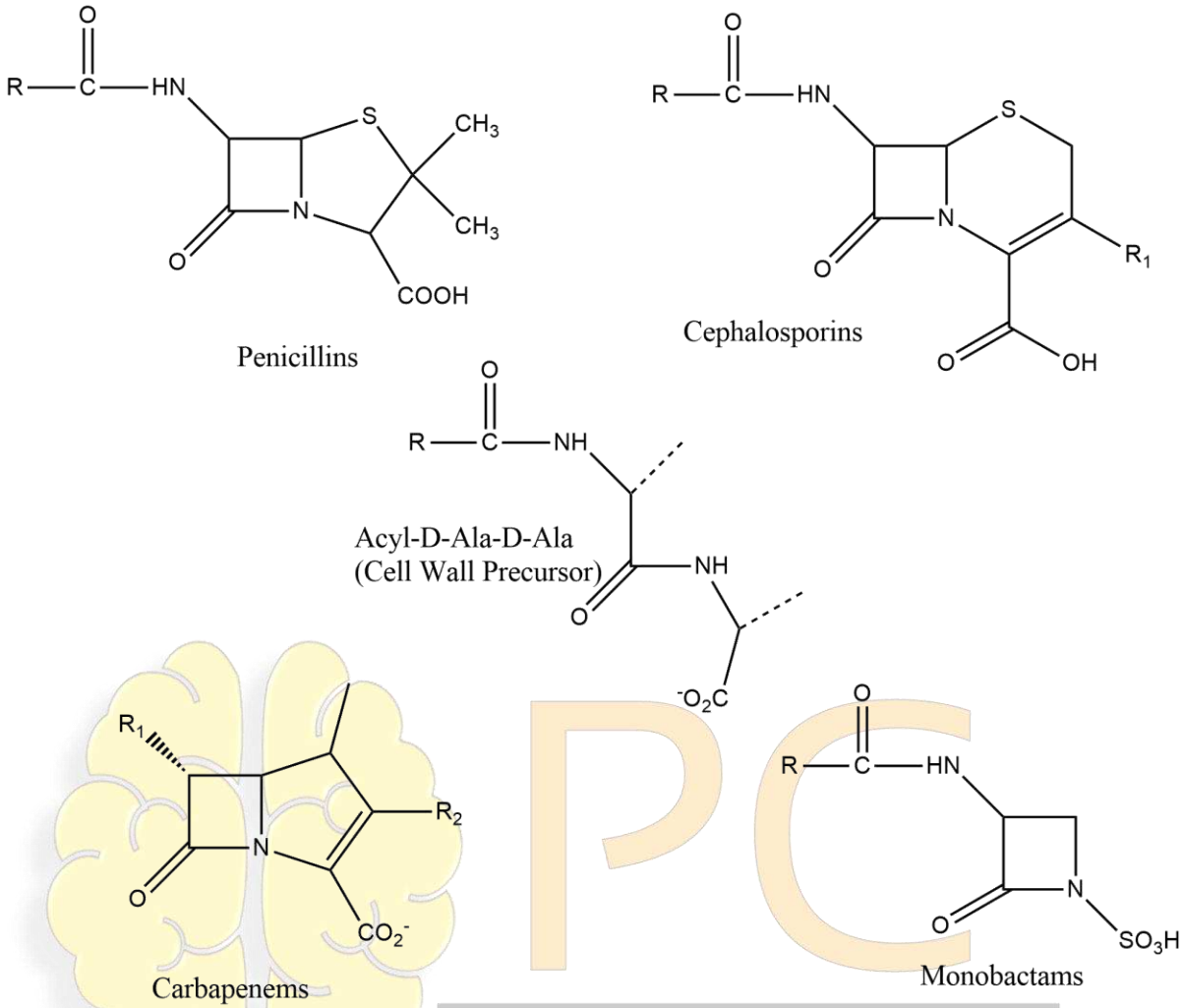
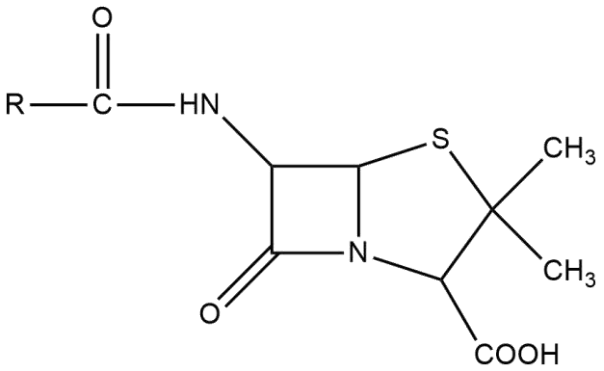


Figure: General Structure of β-lactam antibiotics

2.2. PENICILLINS



2.2.1. History & Developments

- Penicillin, is the most important antibiotic, was first discovered by Scotland's **Alexander Fleming** in 1928 at the Saint Mary's Hospital, London and first antibiotic to be used clinically in 1941.
- **Alexander Fleming** accidently found that bacterial colonies (staphylococcal bacteria) growth is inhibited by co-existing fungal strains or their colonies.
- The mould was identified as ***Pencillium notatum*** and the antibacterial substance was termed as Penicillin by Flemming.
- 1930: Florey and collogue further isolated a compound from the fungus strain and named it penicillin that has antibiotic effects.
- In 1938, Australian scientist HW Florey & team started systematic studies at the oxford university and in May 1940 a crude material available, which was effective against streptococcal infection in mice. Further enough quantity was produced for clinical trilas in 1941. 1st it was administered to a policeman at Oxford who was suffering from staphylococcal and streptococcal infection.
- 1942: 1st clinical trial at the Yale university & the Mayo Clinic. The results was encouraging and dramatic. There was difference between the penicillin made in England and the penicillin produced in America. They were designated as Penicillin-I (Pen. F) and Penicillin-II (Pen. G) respectively.
- Penicillin G could be obtained in pure form and its production began in USA after 1943.
- Penicillin antibiotics are obtained from the mould ***Pencillium notatum*** and ***P. chrysogenum*** (highest yield and employed for the commercial production).

1945: For the contribution Flemming, Floery, and Chain jointly got the Nobel Prize for the medicine.

1943: Sir Robert Robinson at Oxford university determined the correct structure of penicillin.

1945: Later D. Hodgkin's elucidated & confirmed the chemical structure of penicillin by X-ray diffraction.

1957: Sheehan develops the synthetic procedure for production.

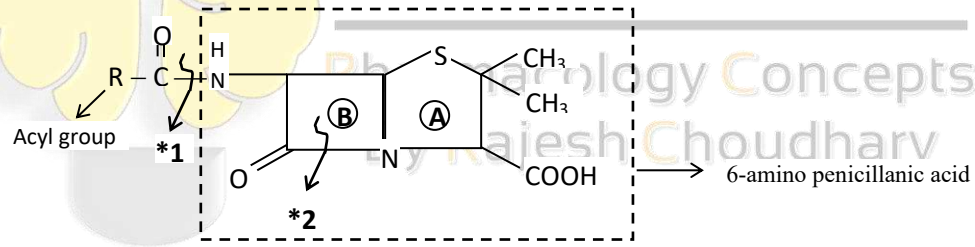
1958: Beechams isolated 6-amino penicillanic acid (6-APA) to use as intermediate for semi-synthetic penicillin derivatives.

1959: British scientists reported the isolation of 6-APA from culture of ***P. chrysogenum*** and this compound can be converted to penicillin by acylation of the 6-amino group.

1971: Sheehan & Ferris develop another procedure for synthetic penicillin.

2.2.2. Structure & Nomenclature

The basic structure of pencillins consists of a thiazolidinering linked to a β-lactam ring (B). The two rings together constitute the basic nucleus 6-aminopenicillanic acid.



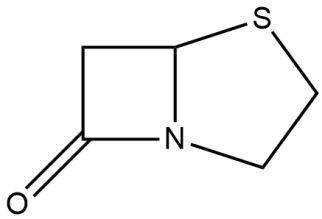
Basic nucleus of penicillin

- ***1**-bond is broken by amidase.*
- ***2**-bond is broken by penicillinase.*

β-lactam ring is strained ring and is sensitive to acid hydrolysis and ***penicillinase*** (*β-lactamase*), which produced by *S. aureus* and further lead to drug resistance.

Stable penicillins have been prepared by minimising the sensitivity of β-lactam ring to acid hydrolysis, ***penicillinase*** and ***amidase***.

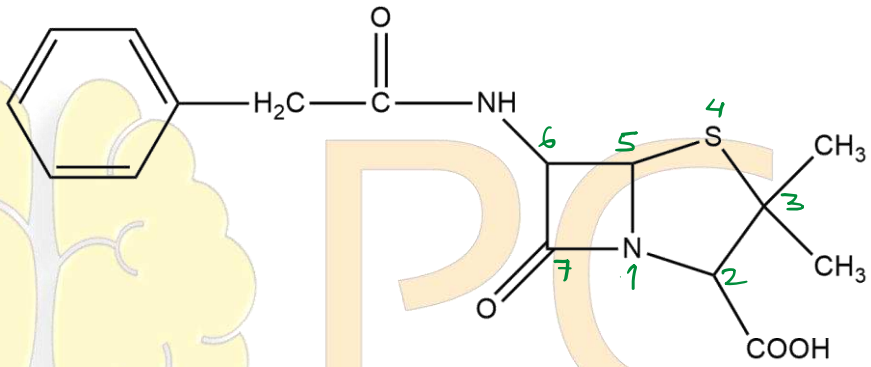
bicyclic skeleton: a β-lactam ring fused with a thiazolidine ring:



(4-thia-1-azabicyclo[3.2.0]heptan-7-one)

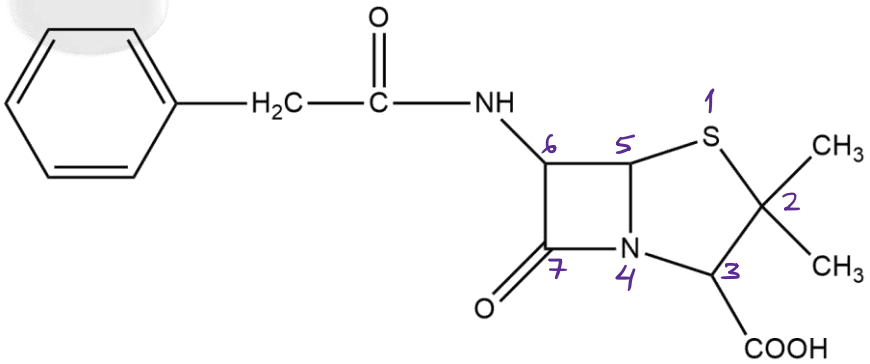
Penicillin

IUPAC or United State Pharmacopoeia; USP system: Aza (N)- 1 & Thia (S)- 4



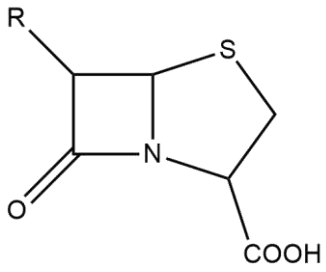
3,3-dimethyl-7-oxo-6-[(2-phenyl acetyl)amino]-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylic acid

(Chemical Abstract System); Aza (N)- 4 & Thia (S)- 1

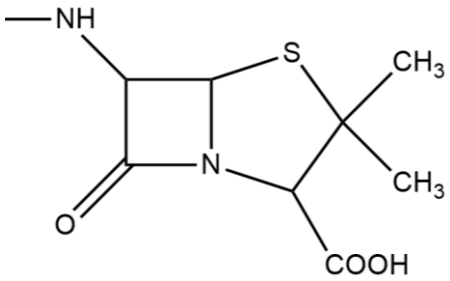


2,2-dimethyl-7-oxo-6-[(2-phenyl acetyl)amino]-1-thia-4-azabicyclo[3.2.0]heptane-3-carboxylic acid

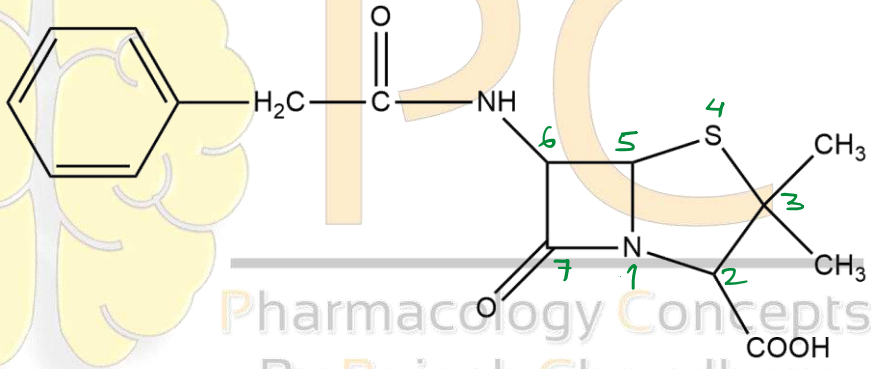
Simplified: Use Penam ring system or penicillanic acid system



Penam



6-amino penicillanic acid



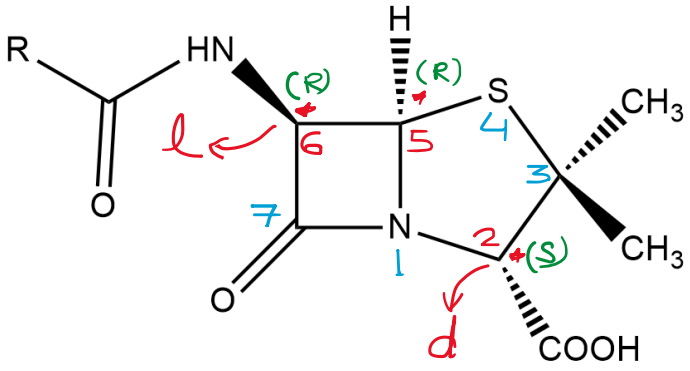
3,3-dimethyl-6-[(2-phenyl acetyl)amino penam -2-carboxylic acid

Or

6-[(2-phenyl acetyl)amino penicillanic acid

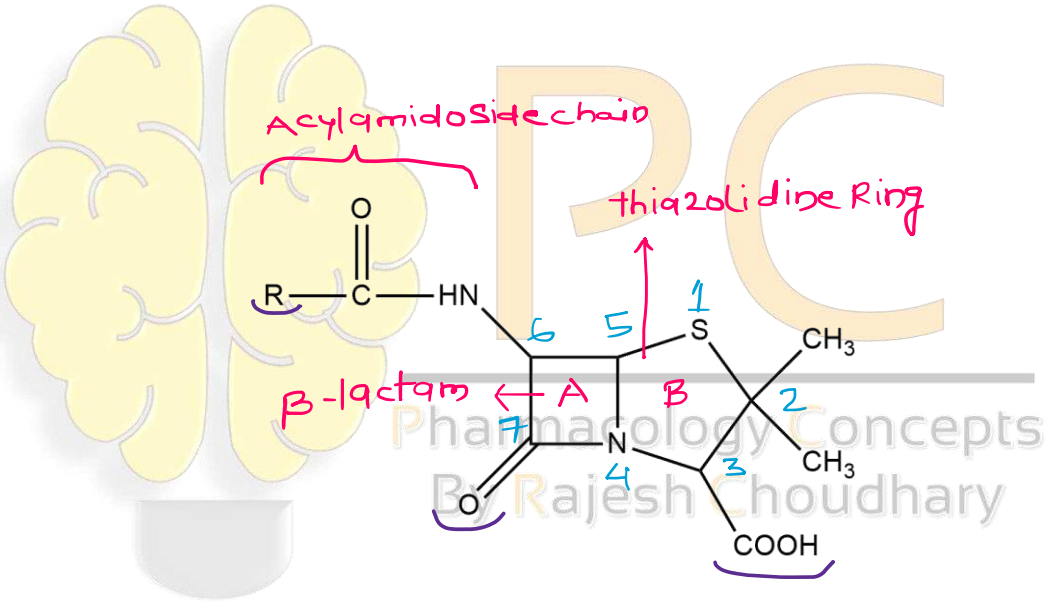
2.2.3. Stereochemistry

- Penicillin has 3 chiral centers at- C-2, C-5, and C-6 (if naming done by USP system)
- **Optical Isomerism**: At C-6- l configuration and at C-2- d configuration. Thus acylamino (at C-6) and carboxylic group (at C-2) are trans to each other.
- **Absolute configuration**: (2*S*,5*R*,6*R*)



(2*S*,5*R*,6*R*) 6-[(2-phenyl acetyl)amino penicillanic acid

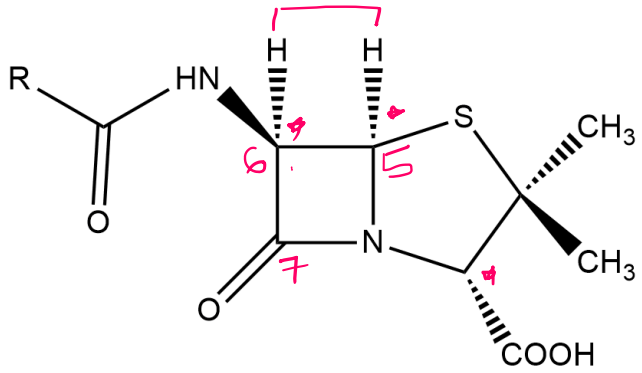
2.2.4. Structural Activity Relationship (SAR) of Penicillin



Basic Ring of Penicillin

1. Intact β-lactam ring and acylamido side chain are essential for antibacterial activity.
2. Side chain of penicillin (-R) is also essential for antibacterial activity, (side chain determines the stability of the penicillin against degradation by acid and *penicillinase*).
3. Cis-form is essential for maximum activity.

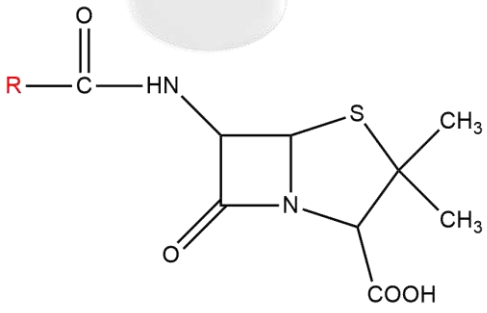
Cis-form

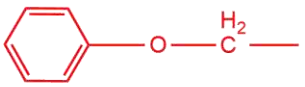


There are 3 major sites for alteration: (-R) side chain, Carbonyl oxygen and -COOH group.

A) -R Side Chain

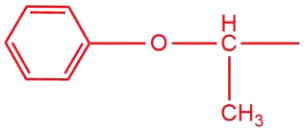
1. Substitution of electron withdrawing group (-NO2, -CHO, -COOR, etc) in the α position of the acyl group increases the resistance to acid hydrolysis and enhance the stability by decreasing the nucleophilicity at carbonyl oxygen. E.g. Phenoxymethyl penicillin (Penicillin V), and Phenethicillin.





Pen.V

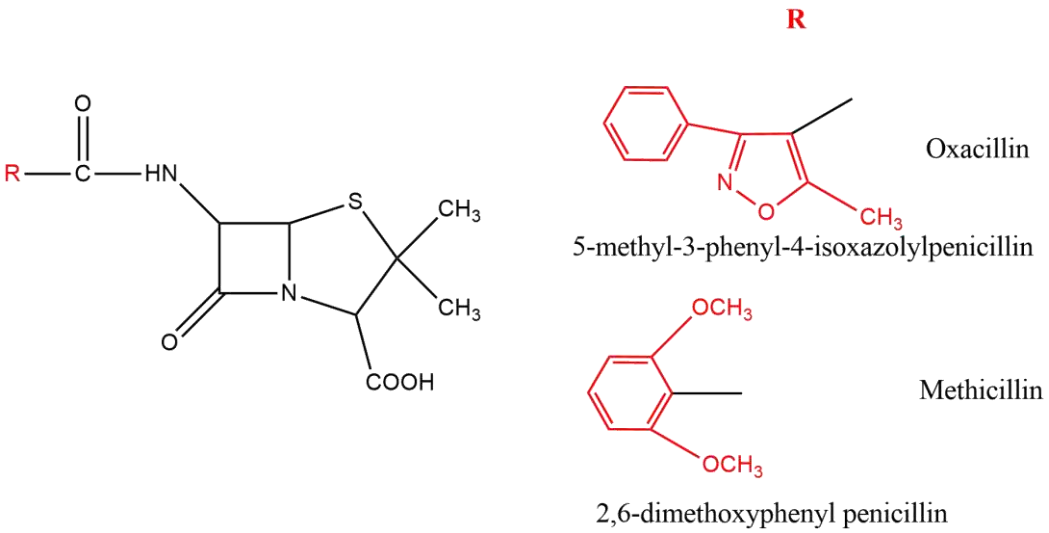
Phenoxymethyl penicillin



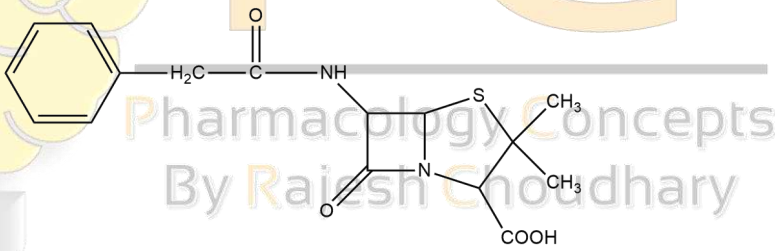
Phenethicillin

Phenoxyethyl penicillin

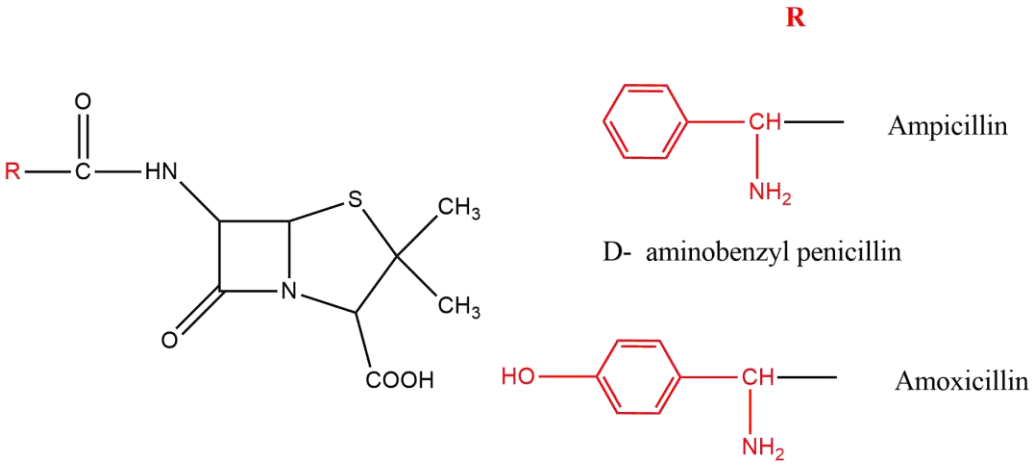
2. Bulky groups (due to steric hindrance) provide the resistance to beta-lactamase. E.g., Methicillin, Oxacillin, Cloxacillin



3. Nature of acylamido side chain helps to determine the plasma protein binding. Lipophilic groups increase the plasma protein binding. E.g., Oxacillin and Cloxacillin have 80-90 % PB while Ampicillin and Amoxicillin have 20-30% PB.
4. The introduction of polar group or ionized molecule into the α-position of the side chain in the benzyl carbon atom of penicillin-G confers against the gram-negative bacilli. Amino, hydroxyl, carboxyl, and sulphonyl increase gram-negative activity. Example: ampicillin and carbenicillin



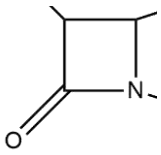
Benzyl penicillin (Pen. G) (Active against Gram +ve Bacteria)



Ampicillin and Amoxycillin (Active against Gram -ve Bacteria)

5. The isomeric forms of penicillin differ in their activity. Example: D-isomer is 2–8 times more active than L-isomer of amoxicillin.

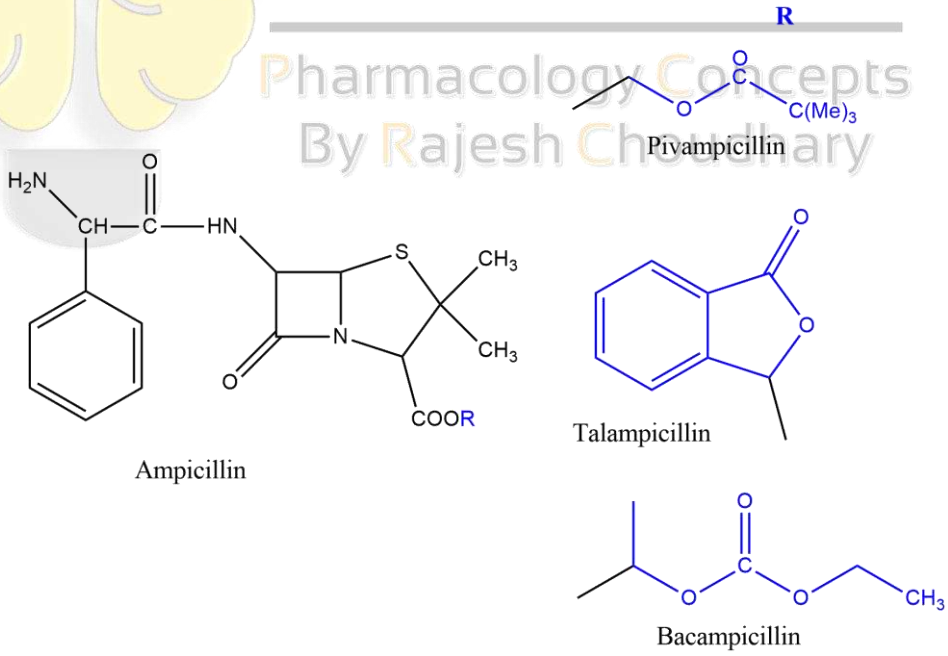
B) Carbonyl Oxygen



1. It is ready for nucleophilic attack, because lone pair of N atom is not involved in the resonance, so it increases the stability of ring and provide better stability and activity.

C) Carboxylic Group

1. Carboxylic acid is important for the activity. It is important to recognize the protein binding site (PBS) located in bacteria and help to penetration in bacterial cell. Change in activity by reduced in alcohol form
2. Ester (-COOR) form used for preparation of prodrugs for enhance the oral absorption. E.g., Esters of Ampicillin.



3. Sodium and Potassium salt are most active

2.2.5. Chemical Degradation of Penicillin

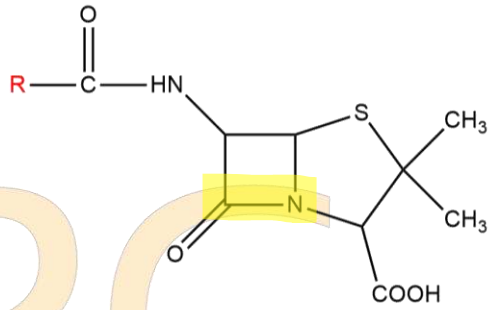
Earlier, penicillin was amorphous in nature therefore required cold temperature for storage.

Later it was purified and converted in white crystalline powder that is stable in dry and normal temperatures for years. But it must be protected from moisture to avoid degradation.

The hydrolysis of penicillin is majorly affected by pH, and enzymes (amidase and beta-lactamase).

Beside these, the beta lactam rings generally susceptible to various

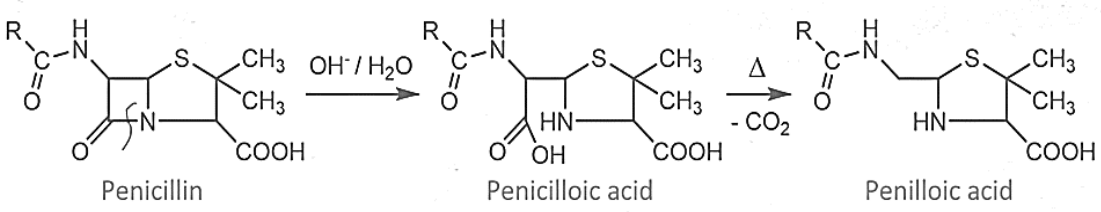
- Nucleophile
- Acid-base reagent
- Metal ions
- Oxidizing Agents
- Solvents (water, ethanol)

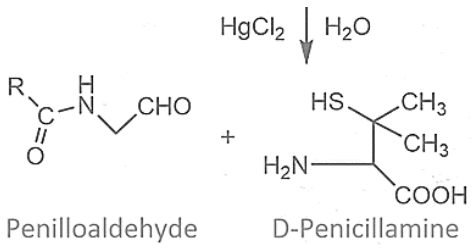


In clinical respect, it is important because allergenicity property of penicillin have shown by its degraded product and formation of penicilloyl protein.

Nature of degradation of penicillin and hydrolytic product mainly depends on the pH.

A) Hydrolysis on Alkaline pH





B) Hydrolysis on Strong Acidic pH (<3)

In strongly acidic solutions (pH < 3), penicillin is protonated at the β-lactam nitrogen, and this is followed by nucleophillic attack of the acyl oxygen atom on the β-lactam carbonyl carbon.

The subsequent opening of the β-lactam ring destabilizes the thiazoline ring, which opens to form penicillenic acid that degrades into two major products penicillamine and penilloic acid. A third product, penicilloaldehyde is also formed.

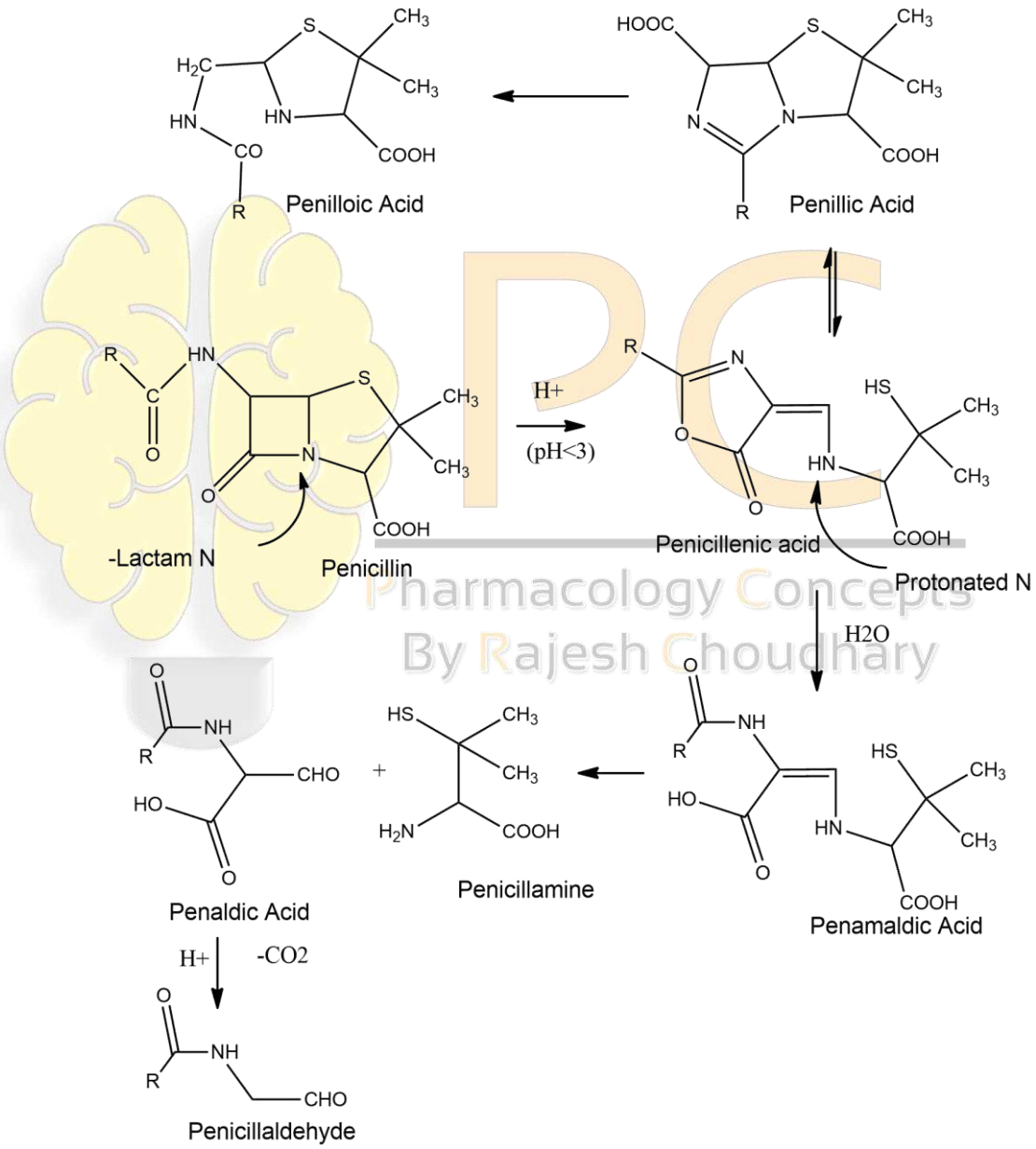
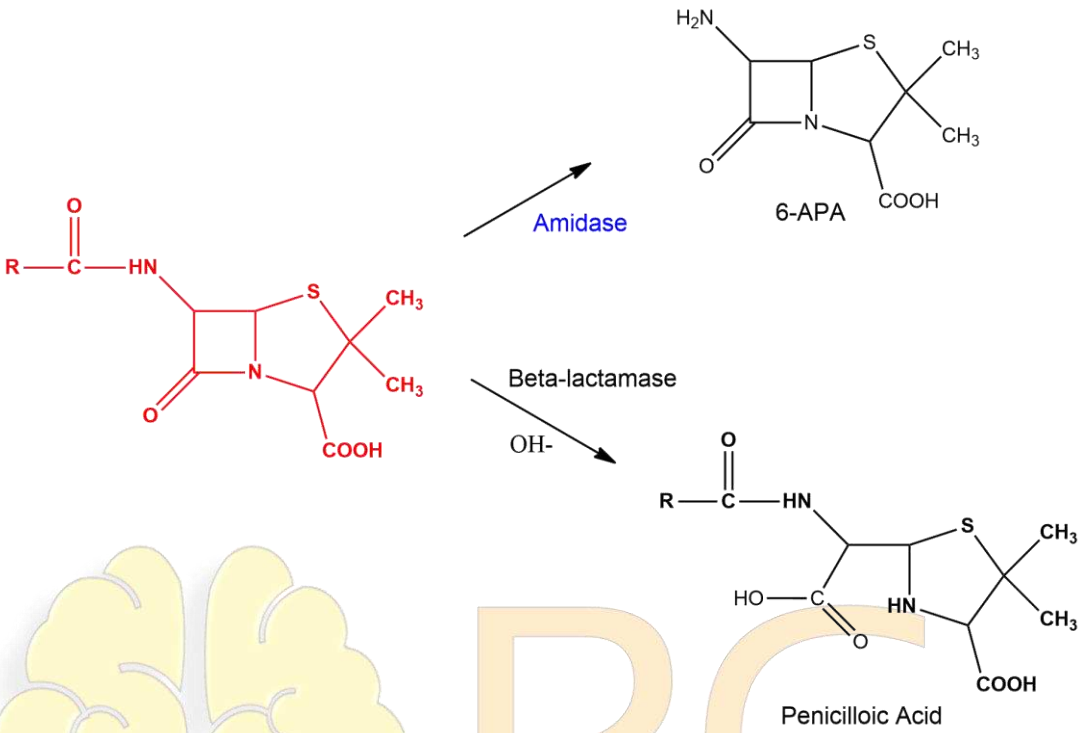


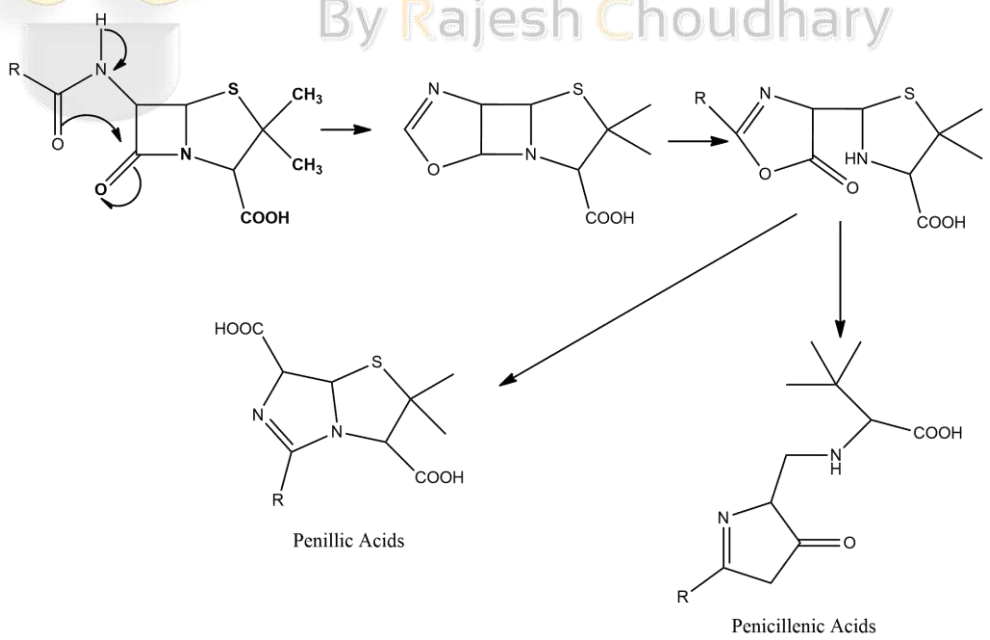
Figure: Degradation of Penicillin in Acidic pH

C) Degradation of Penicillin by Beta-lactamase and Amidase



D) Self-Destruction

Acyl group carbonyl (=O) is electron rich (nucleophile) and able to attack the neighboring carbon of beta lactam (electrophile C). So, it has self-destructive property which can be reduced by using electron withdrawing R group and other nucleophiles, such as hydroxylamine, alkylamines, and alcohol.



2.2.6. Classification of Penicillin

1) Narrow spectrum:

(a) Natural Penicillin: Peni

(b) *Penicillinase and Acid sensitive:* Benzyl penicillin (Pen. G)

(c) *Penicillinase sensitive*: Penicillin V and Procaine penicillin

(d) *Penicillinase resistant:* Flucloxacillin, Cloxacillin, Oxacillin

2) Broad/Extended spectrum:

(a) *Amino-penicillins:* Ampicillin, Pivampicilline, Amoxicillin

(b) *Urido-penicillins:* Azlocillin, Mezlocillin, Piperacillin

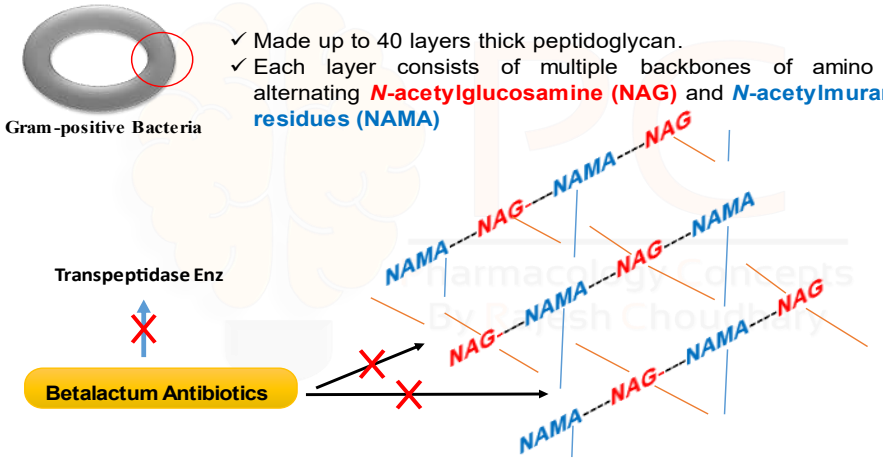
(c) *Carboxy- penicillin:* Ticarcillin, Carbenecillin

*Carboxy and urido-penicillins are prefer for Pseudomonal infection.

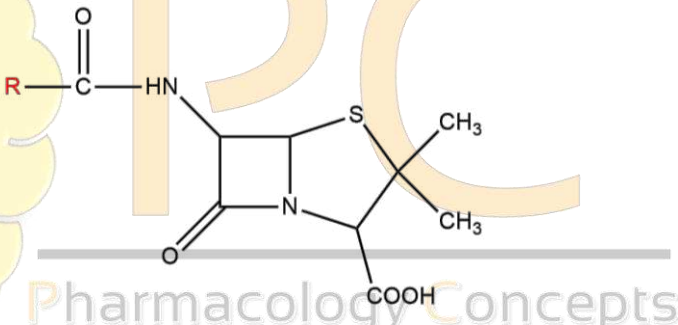
2.2.7. Mode of Action

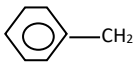
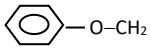
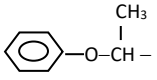
MOA: Penicillins act by inhibiting bacterial cell wall synthesis after attachment to *penicillin binding proteins* present on bacteria. Peptidoglycan is a major structural component of the bacterial cell wall that is cross linked and form a net-like structure. This net like structure provide strength and rigidity to the bacterial cell wall. Penicillins acylate the enzyme *transpeptidase*, inactivating it so that it cannot form a cross link of linear peptidoglycan strands by *transpeptidases* and elimination of D-alanine. This weakens the bacterial cell wall and make the organisms vulnerable to damage by solutes in the surrounding medium (bacteria swell → burst → lysis → death).

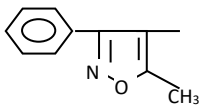
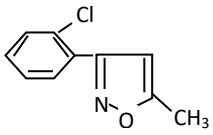
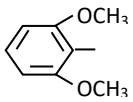
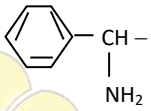
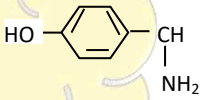
Gram positive bacterial cell wall consists 40 to 50 layers of peptidoglycan whereas *gram negative* bacterial cell wall consists alternative layers of lipoprotein and peptidoglycan. Therefore, Penicillin G limited acts on *gram positive* bacteria and few others.



2.2.8. Important Product and Uses



COMPOUND	R	CHEMICAL NAME	USE
Natural Penicillin			
1. Penicillin G		Benzyl penicillin	Used against Gram positive bacteria like streptococcal, staphylococcal and their infections diphtheria, tetanus, gas gangrene sexually transmitted diseases and anthrax.
Acid Resistant Penicillin			
2. Penicillin –V		Phenoxymethyl penicillin	Similar as Pen.G
3. Phenethicillin		Phenoxyethyl penicillin	Similar as Pen.G

Penicillinase Resistant Penicillin			
4. Oxacillin		5-methyl-3-phenyl-4-isoxazolylpenicillin	Used in the treatment of Penicillin G resistant *Staphylococcus* infection.
5. Cloxacillin		5 methyl-3-(2-chlorophenyl) -4-isoxazolylpenicillin	Used in *penicillinase* producing *Staphyllococcus* infection.
6. Methicillin (*Penicillinase* resistant but not acid resistant ∴ given by parenteral route)		2,6-dimethoxyphenyl penicillin	Used in *penicillinase* producing *Staphyllococcus* infection.
Extended Spectrum			
7. Ampicillin		D-α aminobenzyl penicillin	In the treatment of urinary tract, billiary and intestinal tract, respiratory tract, intections. It is also used in meningitis and other gram +ve and gram –ve infections.
8. Amoxicillin		D-α-amino-p-hydroxy benzyl penicillin	Similar as Ampicillin