



CRUCIAL FACETS OF ADVANCES IN B-LACTAM COMBINATIONS FOR ALLIED MEDICINAL PRACTICES: AN OVERVIEW OF INNATE CHEMISTRY AND BIOLOGICAL PROFILE

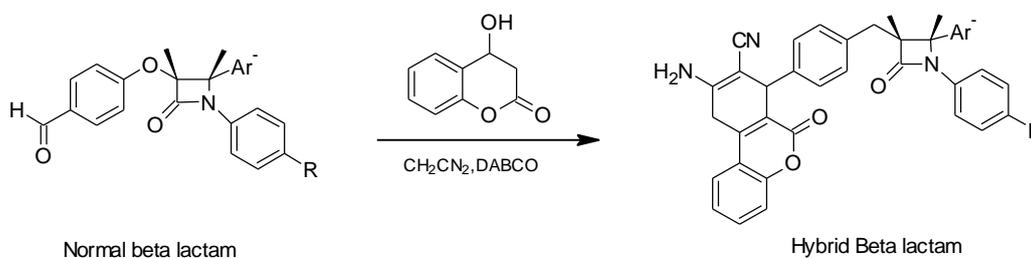
Vilas Katore, Rajendra S. Dongre, Manoj Thakare, Tahir Sheikh

Post Graduate Teaching Department of Chemistry, Nagpur University, Campus, Nagpur-440033, Maharashtra India

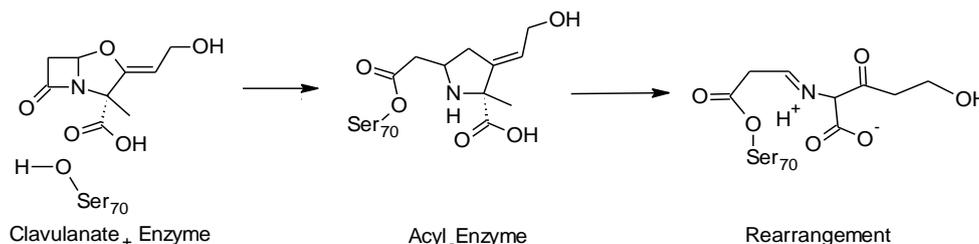
E-mail-vilaskatore10@gmail.com, phone: +91-9689490094

ABSTRACT

The four membered β -lactam are being very important class of organic compounds in medicinal chemistry. Different types of β -lactam antibiotics are basically used to treat the bacterial infections, but novel inventions in β -lactam chemistry has shown the improved bio-activity of it as antiviral, inflammatory, analgesics, Cholesterol absorption agents, antidiabetics, antitubercular, Neuroprotective agents etc. The hybrid β -Lactam is found effective for versatile medicinal applications particularly as an anticancer drugs.



β -Lactamase inhibitor enables β -lactam antibiotics to increase its stability towards its enzymatic hydrolysis caused by bacterial enzymes. This results in efficacious bio-logical activity of β -lactam antibiotics to treat different infections.



A β -lactam innovative research leads transmuted revolution in treatment of bacterial infection, thus saved such diseases suffered millions being an outlining milestone in the history of

mankind. 2-Azetidinone is the one of the simplest β -lactam units of several medicines of bacterial infectious treatments. Till 1970, major research on β -lactams was concerned in domain of antibiotics like penicillins and cephalosporin, nevertheless wide variety of β -lactams were developed for treating varied diseases and antibacterial disorders. Relevant advances in β -lactam combinations for curative practices stressed on emerging novel β -lactam-/lactamase prohibition recipes as comprise of pairing classic antibiotics by new inhibitor to beat bacterial resistance of current drugs which are being effective against broader resistant pathogens than earlier combination. Owing to progressive methodological growths in medicinal chemistry and artificial intelligence, it's conceivable to cultivate varied β -lactam combinations so as to treat diversified diseases or disorders observed in bacterial infections.

KEYWORDS-: β -LACTAM, β -LACTAMASE, BACTERIAL INFECTIONS, MEDICINAL

CHEMISTRY.

1. Introduction-:

A four membered cyclic amide called β -lactam is named due to the nitrogen attachment to carbon atom which is located at β -position with respect to carbonyl group in skeleton.

The innovative β -lactam compounds for their pharmacological activity needs to achieve the following perceptions

1. Wide spectrum of activity
2. Enhanced potency
3. Directed mode of action
4. Development of novel resistance mechanism
5. Grouping Therapies
6. Drug advancement against precise pathogens

The present review paper is well elaborating on the crucial facets of advances in research work on β -lactams/Mixed hybrids/ β -lactamase inhibitors for its different medicinal applications like antibacterial, anti-cancer, anti-viral, anti-diabetic, anti-tuberculosis anti-neurological disorders/diseases etc.

2. Progressive novel resistance mechanism:

Due to the mutagenic nature of pathogens which results by their genetic mutation, pathogens will put resistance against the effects of antibiotic drugs which are designed to target the specified pathogens. Because of this, antibiotics/other medicines are resulting ineffective to treat bacterial infections. This happens commonly with all types of β -lactam drugs due to their strained type of ring which is prone to hydrolysis by bacterial enzymes.

This renders the need to develop novel antibiotic drugs and combination therapy which makes them effective against the muta-genetic pathogens.

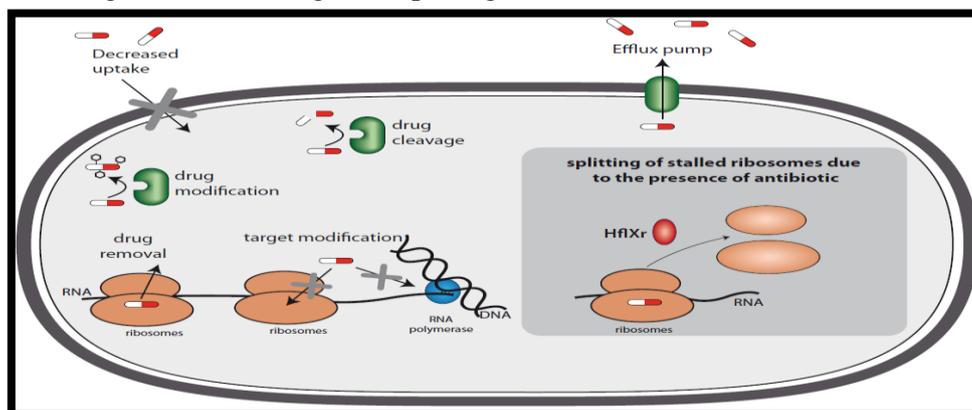


Figure -:1 Development of novel resistance mechanism by bacteria/pathogens

The β -lactam ring constitutes the essential structural foundation for numerous antimicrobial agents including penicillins, cephalosporins, monobactams, and carbapenems. These antibiotics suffer diminished therapeutic effectiveness when bacterial enzymes called β -lactamases cleave their β -lactam rings through hydrolytic processes, rendering these antimicrobial compounds ineffective and allowing pathogenic bacteria to develop resistance mechanisms. According to structure-activity principles, β -lactam compounds demonstrate antimicrobial properties by structurally resembling the natural d-Ala-d-Ala dipeptide substrate, which normally interacts with enzymatic targets known as penicillin-binding proteins (PBPs). These proteins facilitate cross-linking reactions within the peptidoglycan matrix of bacterial cell wallsⁱ

Hermann Staudinger achieved the initial synthesis of β -lactam antibiotics in 1907 through a [2+2] cycloaddition methodology involving a Schiff base derived from aniline and benzaldehyde reacting with diphenylketone. β -lactam antimicrobials function by disrupting bacterial transpeptidase catalytic processes through interaction with PBPs, with each bacterial strain containing minimally four distinct PBPs. Bacterial classification into gram-positive and gram-negative categories depends on morphological characteristics and cell wall structural composition.ⁱ⁻ⁱⁱ

Gram-positive bacteria possess cell walls with abundant peptidoglycan content, creating thick and inflexible cellular barriers compared to gram-negative counterparts. Low molecular mass β -lactam antibiotics can readily traverse the cytoplasmic membrane's outer layers to reach PBPs or transpeptidase enzymes. Conversely, gram-negative bacteria feature thinner cell walls where minimal peptidoglycan layers are enclosed between inner and outer membrane structures. The outer membrane functions as a molecular barrier, preventing periplasmic protein loss while restricting hydrolytic enzyme penetration, thereby blocking certain antibiotic access.

The outer membrane architecture includes phospholipids, lipoproteins, and lipopolysaccharides, with lipopolysaccharides representing crucial components of gram-negative bacterial species that influence biological activity and trigger inflammatory responses. Lipopolysaccharides comprise core polysaccharides containing 6, 7, and 8-carbon sugars along with O-polysaccharides containing 6-carbon sugars. The O-polysaccharide component serves as a significant antigenic determinant.

Protein channels termed porins exist within the outer membrane, consisting of protein subunits that assemble to create membrane pores forming small channels permitting diffusion of low molecular weight substances including nutrients and hydrophilic antimicrobials. Molecules exceeding 600 Da cannot traverse these channels effectively. Porins limit antimicrobial agent access to gram-negative bacterial cytoplasm. For therapeutic efficacy, antimicrobial compounds must successfully penetrate both the outer membrane and cytoplasmic membrane of gram-negative bacteria before entering the cytoplasm. Consequently, gram-negative bacteria demonstrate greater resistance to antibacterial agents compared to gram-positive bacteria.

Nobel Prize winner Woodward established parameter 'h' as a measurement of trigonal pyramid height defined by nitrogen and its three neighboring atoms, where 'h' correlates with β -lactam bond strength. Reduced 'h' values indicate enhanced β -lactam bond stability and decreased compound reactivity. Various β -lactam compounds exhibit different 'h' values reflecting their structural characteristics.

Monobactams - 0.05 to 0.10 Å

Cephems - 0.20 to 0.25 Å

Penams - 0.40 to 0.50 Å

Carbapenems

& Clavams - 0.5 to 0.60 A0

This indicates that Carbapenems and Clavams are being more reactive towards hydrolysis and this is due to weaker β lactam bonds.

After 1990, because of advances in medical science and chemistry, Scientists found the use of β -lactams to treat other type of diseases/disorders and can be used as anticancer, cholestrol absorption inhibitor, antidiabetic, anti-inflammatory, antagonist activity, CNS drugs, antiparkinson disease, anti-HIV etc apart from their regular antibacterial activity. New type of β -lactams are possessing broad range of activity spectrum with specific resistance mechanism

3.0 Contribution of different antibacterial agents in bacterial infection treatment:-

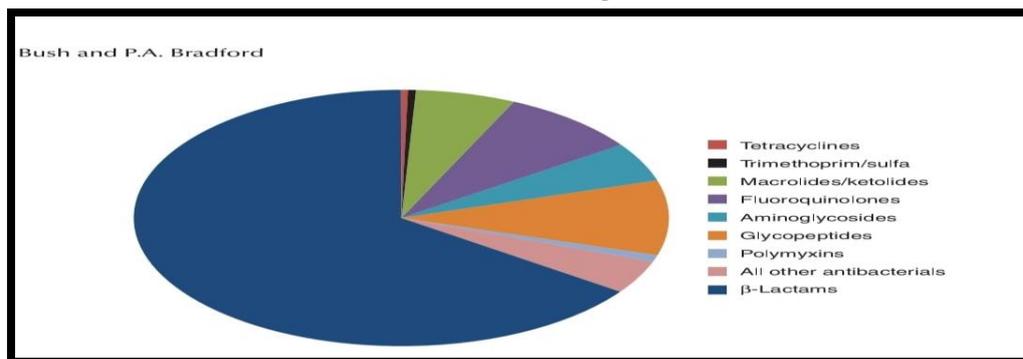


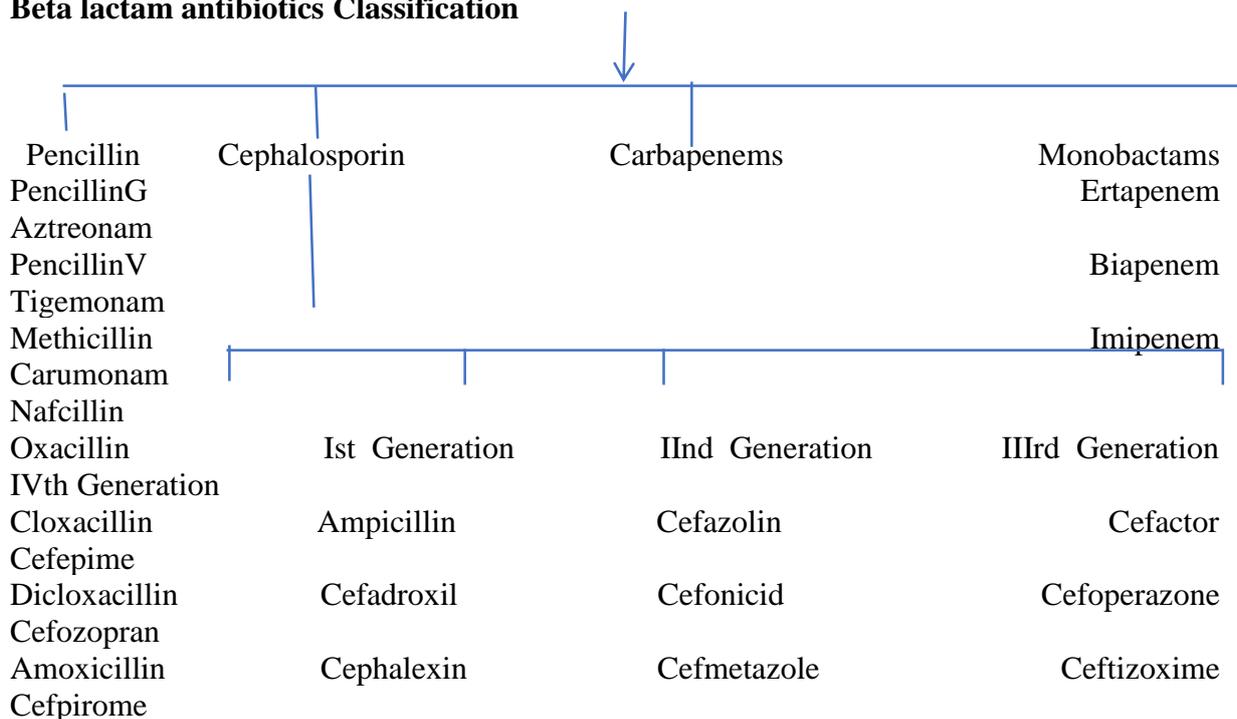
Figure-2: Contribution of different antibacterial agents

In USA, almost 65% of prescriptions by doctors are of β -lactam drugs out of which are from cephalosporin family. [Source- Data from the IMS MDART Quarterly Database on file at AstraZeneca.].

4.0 The classification and pharmacological activities of β -lactams:-

β -lactam antibiotics are classified based on the chemical nature of ring that is fused with β -lactam pharmacophore unit forming a non-coplanar structural unit offering a pharmacological activity. 2-Azetidinone is being simplest form of β -lactam.

Beta lactam antibiotics Classification



Carbenecillin	Cephaprin	Cefotetan	Ceftazidime
Cefquinome			
Ticarcillin	Cephradine	Cefuroxime	Cefixime
Ceftobiprole			
Piperacillin		Cefamantadole	Ceftriaxone
Ceftaroline			
Meziocillin			Moxalactam
Fosamil			
Aziocillin			

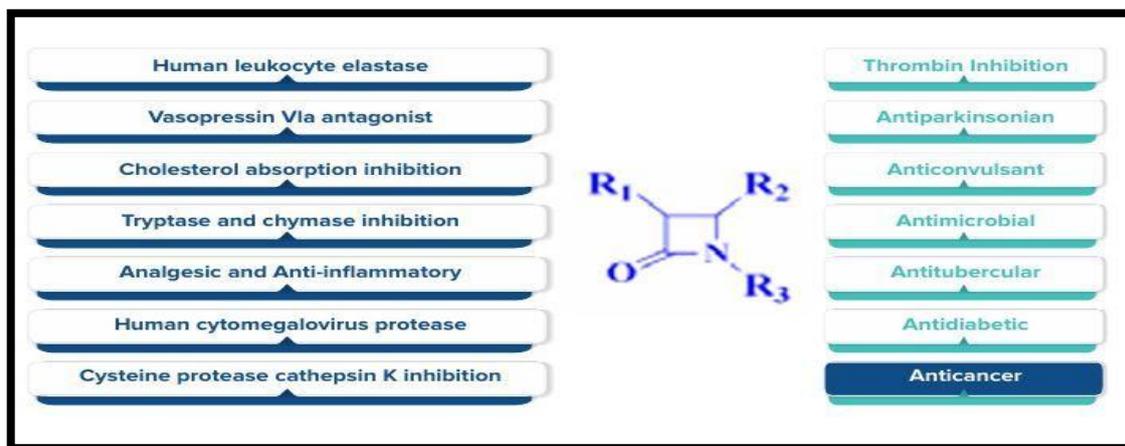


Figure-3: The pharmacological activities of β -lactams

5.0 Exploration of recent developments of β -Lactams compounds for medicinal applications

5.1 β -Lactams for anticancer activity:-

Currently a major threat to human life is of cancer disease and medicinal scientists are putting their efforts to develop an effective remedy for the same. β -lactams are seeming to work effectively against uncontrolled malignant cell growth which is causing the interference of normal physiological function and lifestyle of human being. Its main focus is to inhibit the tumour cell growth and induction of apoptosis in which a cell undergoes self-suicide without affecting the adjoining cell in the malignant cell population.ⁱⁱⁱ

According to research work of Ojima, 2-Azetidones can be used as a useful intermediate in organic synthesis. Considering ideality of β -lactams as building blocks, medicinal research Scientists developed the research strategies to synthesize the racemic and asymmetric nitrogen containing multifunctional compounds which on its decomposition produces various heterocycles like three, four, five, six and medium sized heterocycles, Hydroxyl amine acid derivatives, β -amino ketones-amino alcohols etc.

2-Azetidinone serves as a crucial building block in the preparation of complex therapeutic compounds, including the macrolide anticancer agent lankacidin C and various paclitaxel derivatives used in oncology treatments. Multiple synthetic strategies have been developed to construct β -lactam rings with precise stereochemical control.^{xxxv, lxxxi, lxxxii, lxxxv.}

Zinc-catalyzed Reformatsky condensation of imines using titanocene dichloride as a promoter provides one effective route. Thermal [2+2] cycloaddition between ketene species and imine substrates offers another reliable method. The preparation of α -halogenated β -lactams from α , β -unsaturated amides through a modified Hunsdiecker-type decarboxylation represents an alternative approach. Radical-mediated cyclization of enamide precursors employing cerium (IV) ammonium nitrate generates diversely substituted β -lactam products. Asymmetric

Staudinger cycloaddition utilizing ketenes and various imine derivatives enables enantioselective synthesis. Copper-catalyzed Kinugasa coupling of terminal acetylides with nitrones provides access to β -lactam scaffolds. Highly stereoselective intramolecular C-H insertion of α -methoxycarbonyl- α -diazoacetamides followed by ring closure yields enantiomerically enriched β -lactam products. These methodologies collectively furnish synthetic chemists with diverse tools for accessing stereo chemically defined four-membered lactam rings essential for pharmaceutical development.^{xlvi}

In order to develop more potent cytotoxic agent, medicinal scientists has developed new approach of chemistry known as molecular hybridization. This type of research strategy is very effective for the synthesis of innovative compounds with improved antiproliferative activity as compared with parent molecules resulting in less drug-drug interactions & minimises drug resistance. Novel anticancer drugs are being synthesized by linking β -lactam compounds with different biologically active moieties with anticancer properties.

Anticancer β -lactam hybrid compounds have been successfully developed through molecular hybridization approaches, incorporating various pharmacologically active moieties. These include combinations with thymidine 1, 2, 3-triazole structures, chalcone derivatives, retinoid compounds, isatin fragments, and benzimidazole units.

The incorporation of thymine-based heterocycles in these hybrid molecules demonstrates remarkable potential for cancer treatment due to thymine's fundamental role as a nucleotide component in genetic material. Since thymine serves as a critical building block in both deoxyribonucleic and ribonucleic acid structures, its presence in hybrid compounds can effectively interfere with essential cellular processes involved in tumor growth and proliferation.

Through strategic molecular hybridization methodology, researchers synthesized novel compounds incorporating thymine moieties linked to β -lactam scaffolds. These newly designed molecules underwent comprehensive cytotoxicity evaluation using multiple human malignant cell lines including L1210 leukemia cells, CEM T-lymphoblastic cells, and HeLa cervical carcinoma cells. The experimental results revealed differential antiproliferative effects across the tested cell lines. While the compounds exhibited potent growth inhibitory activity against both L1210 leukemia and CEM T-cell populations, the cytotoxic response in HeLa cervical cancer cells showed comparatively reduced efficacy. This variation in cellular response patterns suggests that the thymine- β -lactam hybrids may possess selective mechanisms of action that preferentially target specific types of malignant cells, particularly those of hematopoietic origin compared to solid tumor-derived cell lines.

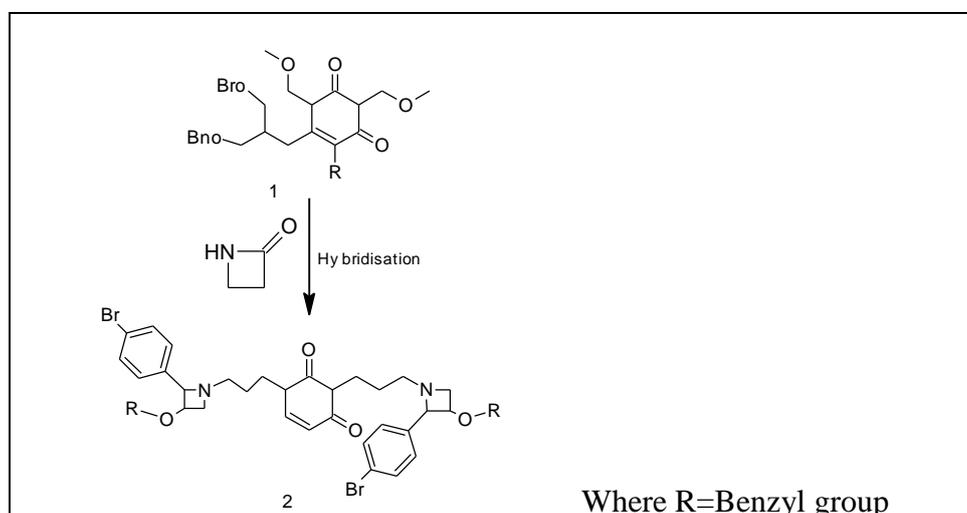


Figure-:4 Thym1, 2, 3-Triazole-β-lactam hybrids

Structure Activity Relationship

1. R - Bn, displayed the best antiproliferative result against CEM cells (IC₅₀ = 3.4 μM).
2. Good selectivity between CEM cells and Hela cells
3. Substituents on phenyl ring play an important role

Many new medications with antiproliferative properties have been created and synthesized with the aid of the molecular hybridization concept. For example, a number of novel 1, 4-diaryl-2-azetidines of combretastatin A-4 (CA-4) with antiproliferative, anti-apoptotic, and tubulin polymerization-inhibiting properties^{vi-vii} Vinca alkaloids, which target microtubules, and paclitaxel are the most effective chemotherapy drugs.^{vii} Colchicine functions as a tubulin stabilizing agent by binding to the alpha and beta tubulin subunits to form a colchicine-tubulin complex.^{ixiv} (1, Fig-6). This complex on depolymerisation produces a heterodimer.

Colchicine is a tubulin stabilizing agent that forms a colchicine-tubulin complex by binding to the alpha and beta tubulin subunits^{vi}

By preventing tubulin assembly, it results in mitotic arrest and cell death. The colchicine is firmly linked to the tubulin site.^{vii, x} Compound CA-4's low water solubility, low bioavailability, volatility, and susceptibility to clearance limit its application as an anticancer drug. Clinical trials are presently being conducted on fosbretabulin, a water-soluble prodrug of CA-4 that exhibits strong vascular action.^{ix} Clinical evaluations are also conducted on the phosphate prodrug of combretastatin A-1 (2C) and a serine prodrug of ombrabulin. Numerous chemically modified, double-bond shaped, and substitution-patterned analogues of compound CA-4 have been created and their activity assessed in clinical settings. Many colchicine binding site inhibitors, including CA-4, CA-1, podophyllotoxin (3a), phenastatin (3b), and 4-benzoyl imidazole (3c), share the structural characteristic of 3,4,5 trimethoxy phenyl.^x are also prepared and clinically evaluated.

Researchers have created CA-4 analogues with strong antitumor efficacy by using the beta lactam scaffold. Analogues of Chiral-β-lactam bridged CA-4 are among the most effective antitumor compounds.^{ix, xiii} The β-lactam analogues of this series, including 5a and 5b (fig. 6), have strong antiproliferative properties against leukemia cell lines and human breast cancer MCF-7 and MDA-MB-231^{ix, xiii}. According to scientific research, molecule CA-4 and its analogues can be directly glucuronidated by UGT at ring B's meta-hydroxy position, which inactivates HT-29 cells.^x The antiproliferative activity of CA-4 has been increased in combretastatin-refractory HT-29 cells following the removal of the metahydroxy B ring. The rigid analogues of CA-4, 1, 5-diaryltetrazole and 4, 5-disubstituted oxazole, which lack the ring B metahydroxy group, revealed a similar kind of outcome.^{iv & xiii}

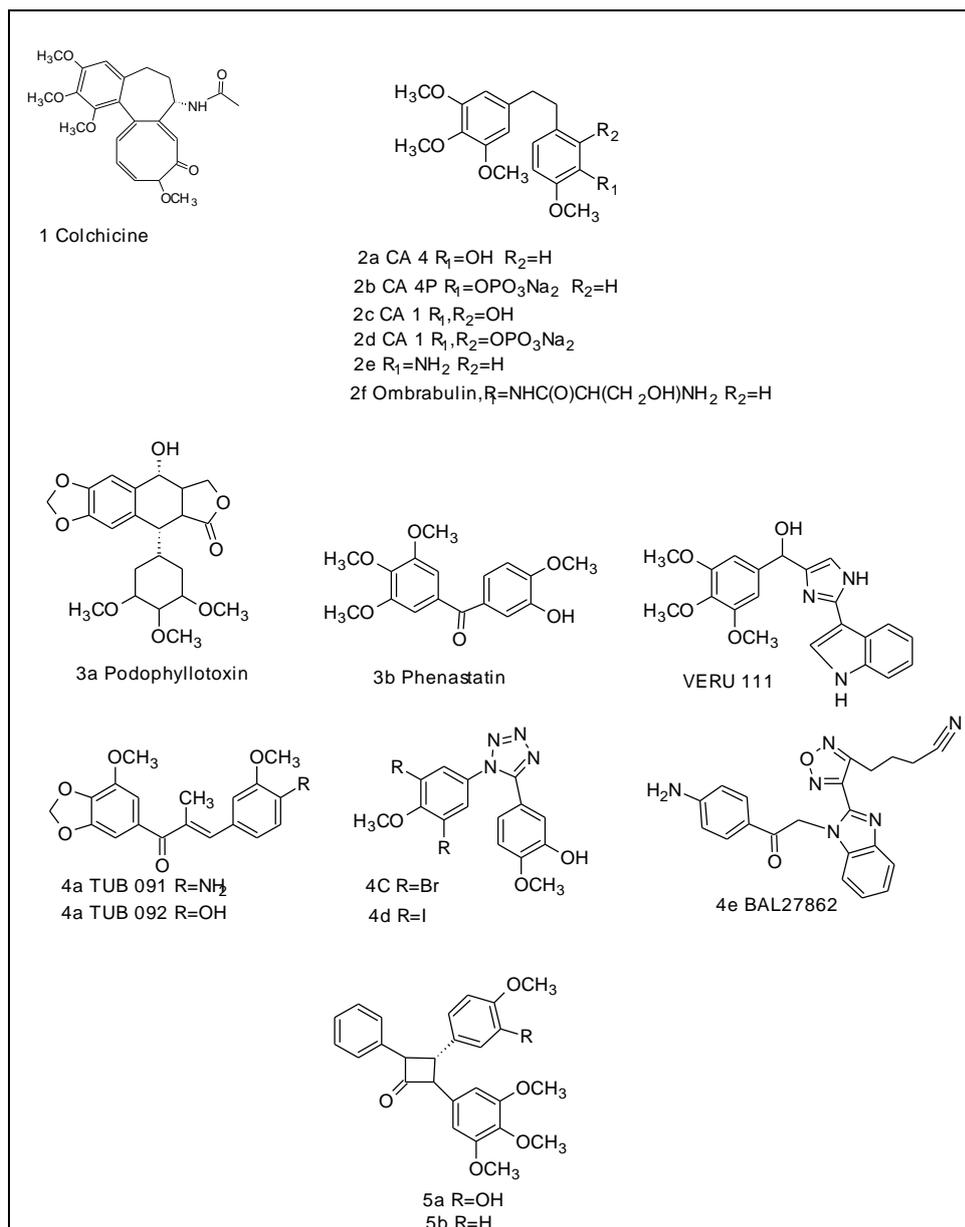


Figure-5: Chemical structures of colchicine, CA-4 and related colchicine-binding micro tubule-destabilising agents.

5.1.1 Chemistry

The development of β -lactam molecular structures as hybrid compounds involves sophisticated synthetic approaches that yield products with enhanced biological properties. Through careful optimization of reaction conditions, researchers have successfully created diverse β -lactam derivatives with varying substitution patterns and stereo chemical configurations.^{vi} This was being confirmed by H^1 NMR

Initial formation of imine intermediates occurs through direct condensation reactions between selected amine precursors and corresponding aldehyde compounds. These reactions proceed efficiently in refluxing ethanol solvent systems, consistently producing high-yield imine products that serve as key building blocks for subsequent transformations. The resulting imine compounds demonstrate excellent stability and reactivity profiles suitable for further synthetic manipulations.

The construction of β -lactam ring systems utilizes an adapted version of the Staudinger cycloaddition methodology, which provides exceptional stereoselectivity favoring trans-configured products exclusively. This stereochemical preference proves advantageous since trans-isomeric β -lactam compounds consistently exhibit enhanced biological activity compared to their cis-counterparts. Spectroscopic characterization through proton nuclear magnetic resonance confirms the exclusive formation of desired stereoisomers throughout the synthetic sequence.

Alternative synthetic pathways employ environmentally conscious methodologies, particularly microwave-enhanced Reformatsky reactions that generate unsubstituted β -lactam frameworks efficiently. These green chemistry approaches minimize solvent usage while maintaining excellent product yields, representing sustainable alternatives to traditional synthetic methods. The microwave assistance significantly reduces reaction times while improving overall process efficiency.

Functional group modifications of initial β -lactam products enable access to diversely substituted derivatives. Treatment of acetoxy-substituted precursors with sodium bicarbonate or hydrazine reagents generates corresponding hydroxyl-functionalized products through mild hydrolysis conditions. These transformations proceed cleanly without affecting the core β -lactam ring integrity.

For amino-substituted derivatives, specialized reagent systems including Mukaiyama's chloromethylpyridinium iodide facilitate selective transformations while preserving stereochemical purity. These conditions ensure exclusive trans-isomer formation, maintaining the beneficial stereochemical characteristics required for optimal biological performance.

Comprehensive stability evaluations under physiologically relevant conditions provide crucial information regarding potential pharmaceutical applications. Selected representative compounds undergo systematic degradation studies across varying pH environments, simulating different biological compartments. Extended incubation periods at physiological temperature reveal distinct stability profiles among different structural classes.

Analytical monitoring through high-performance liquid chromatography enables precise quantification of compound degradation over extended timeframes. Results indicate significant variations in stability depending on structural features and environmental conditions. Certain derivatives demonstrate exceptional stability with half-lives exceeding fifteen hours under most conditions, while others show pH-dependent degradation patterns with reduced stability under alkaline conditions.

These stability characteristics directly influence the potential therapeutic utility of different β -lactam structural classes, with more stable derivatives offering advantages for pharmaceutical development. The correlation between structural features and stability profiles provides valuable guidance for designing optimized β -lactam therapeutics with improved pharmacological properties

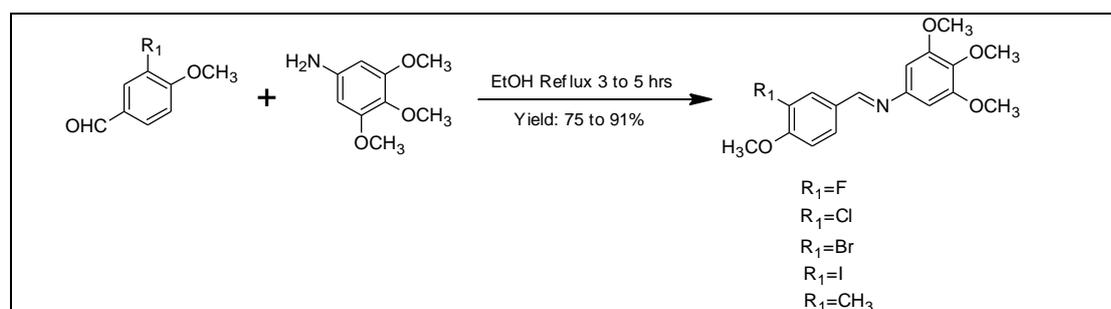


Figure-6 Synthesis of Imines agents (Schiff's Base)

moieties both demonstrate diverse biological properties including antimicrobial, antifungal, and antitumor activities, along with additional therapeutic effects. Through pharmacophore hybridization strategies, enhancement of various characteristics such as therapeutic efficacy, pharmacokinetic profiles, and mechanisms of action becomes achievable. A stereo selective ketene-imine cycloaddition process involving 2-(4-formylphenoxy) acetic acid and imine substrates, conducted in the presence of triethylamine as an organic base and p-toluenesulfonyl chloride, yields β -lactam products. These intermediates subsequently undergo multicomponent reactions with chromene precursor molecules using triethylenediamine as a catalyst to generate β -lactam hybrid compounds.

Biological evaluation encompassed both anti-inflammatory and anticancer screening of aldehyde-functionalized β -lactam derivatives. The chromeno- β -lactam conjugates exhibited promising anti-inflammatory profiles for both compound series. The observed activity demonstrates correlation with varying substituent patterns at the C1 and N1 positions of the β -lactam framework, indicating structure-activity relationships that influence therapeutic potential.

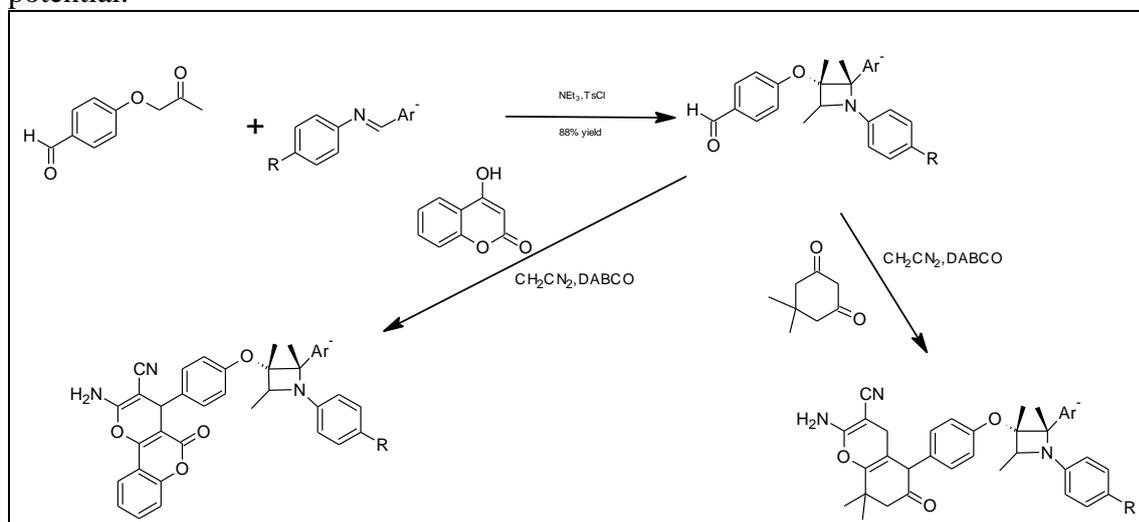


Figure-8: Synthesis of hybrid β -lactams from aldehyde β -lactam and Chromens

5.4 β -Lactams as Cholesterol Absorption Agents:-

The research study on β -lactam compounds has revealed its activity as a Cholesterol absorption inhibitor. Atherosclerotic coronary heart disease (CHD) has been the leading cause of death due to lifestyle changes and increasing serum cholesterol levels in the population. Beta-lactams are also used as potential and highly efficacious cholesterol absorption inhibitors. Recent research focuses on the potential of antibiotic drugs to treat cancer (e.g. bleomycin). A second-generation β -lactam antibiotics viz. Ezetimibe a key structural elements of active metabolites. The studies of Ezetimibe on its mechanism of action was helpful to develop the many biochemical tools for the investigation and identification of novel proteins involved in cholesterol uptake^{xiv}.

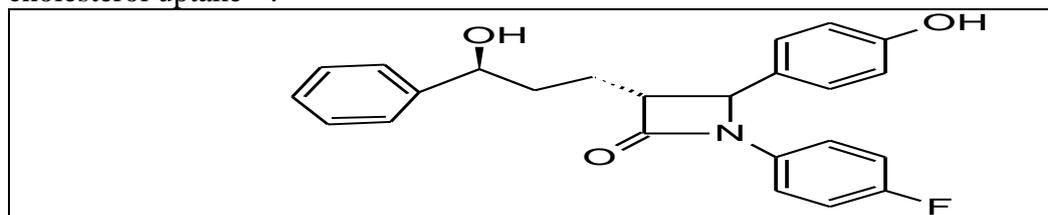


Figure-9: Ezetimibe as a Cholesterol absorption inhibitor

5.5 β -Lactams for anti-diabetic activity:-

A metabolic disorder resulted due to defect in insulin secretion and action which causes increase in blood glucose level is known as diabetes mellitus (DM). Insulin deficiency causes chronic hyperglycaemia by disturbing carbohydrate, fat & protein metabolism and consequently affecting normal physiological activity cycle^{lxiii-lxiv}

The recent scientific study and its subsequent clinical study on β -lactam compounds prepared by various methods has revealed to work as potent anti-diabetic drugs. During pandemic covid 19 period, there was high risk of covid infection to diabetic patients. Diabetic patients are susceptible to acquire multiple life threatening complications like kidney failure, lower limb gangrene^{lxiv}. It can also cause heart attack and strokes in case of timely non control of diabetic. The patients suffering from diabetic are susceptible for respiratory tract infections, urinary tract infections, skin and soft tissues infections, diabetic foot ulcers, otitis and periodontal infection^{lxiv}. As per the survey on diabetics by WHO, there is increase of 8.7% in 2014 from 6.5% in 1980 which has resulted due to unbalanced diet habits of generation.

Generally non-steroidal metformin drug is used to treat the diabetes. β -lactams can act as hypoglycaemic agents.

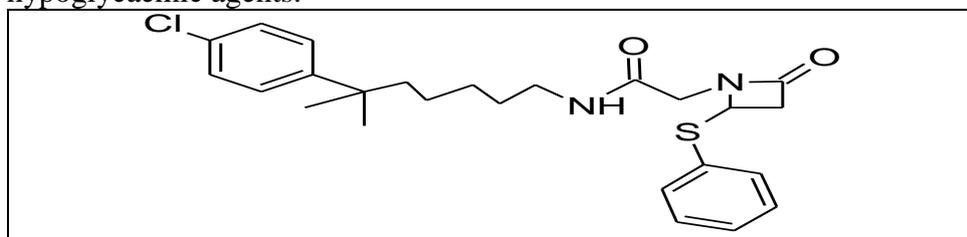


Figure-10: Anti- hyperglycaemic activity shown by monocyclic beta lactam compound

Azetidinones have not been reported as exclusive anti-hyperglycaemic agents until date. There is a report of compound A of this activity along with other activities such as anti-atherosclerotic, anti-anginal, anti-hypertensive etc.

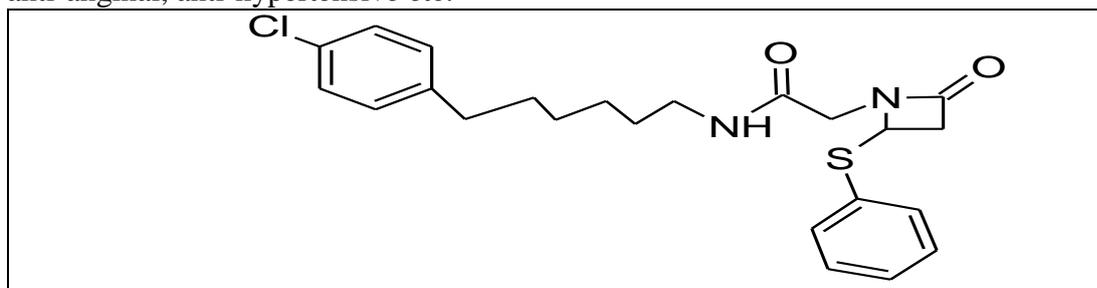


Figure-11: Anti-hyperglycaemic activity of compound A

The structure activity relationship (SAR) study for its antidiabetic activity of the said test compound revealed the impact of substitution for its anti-diabetic activity.

- The phthalimido substitution at C3 has shown the best antidiabetic activity followed by phenyl and phenoxy substitution. The substitution by 1, 3 butadienyl has resulted total loss of antidiabetic activity.
- The substitution of N1 by cyclohexyl and isopropyl is found to be more active than its substitution by phenyl and para methoxy phenyl group.
- The substitution of C4 by styrel and paramethoxy phenyl group is offering better antidiabetic activity than its phenyl substituted analogues.

The structure activity relationship (SAR) study of above compound shows that it can work as efficacious anti-hyperglycemic activity based on its substituents^{iv}. The increased anti-

hyperglycemic activity is due to increased insulin activity or generation of glycogen synthetase enzyme^{liii}

5.7 Neuroprotective Properties of β -lactam compounds-:

The main neurotransmitter present in the central nervous system of all mammalian living organism is Glutamate which plays an important role in several physiological process like memory, learning or pain .Excitotoxicity is being an abnormal effect in neuronal excitation resulted due to deregulation of glutamate^{xviii}.This is generally observed in case of many pathological conditions like stroke,epilepsy,multiple Sclerosis, Alzheimer's disease and Parkinsons disease etc.The Glutamate is getting metabolized into glutamine after its removal from the synaptic cleft by astrocytes^{xx}.Number of glutamate transporters are present in central and peripheral nervous system. But among them the most important are being GLT1/EAAT2, GLAST, and EAAT1 which are combinely uptaking more than 95% of Glutamate in adult forebrain. β -lactam compounds are potentially used to treat the dysfunctioning of neurotransmitter and gets rid of several brain diseases^{xiv}.

Beta lactam antibiotics ceftriaxone is able to avoid the cell death in cultured neurons depriving of oxygen and glucose which can prevent cellular hyper gliosis and loss of muscular strength in a knock out muscular a mouse model. The neuroprotective effect of β -lactam compounds is because of their ability to stimulate the expressions of GLT1 and regularizing the Glucamate concentration in synaptic cleft ^{xxi}.

5.8 β -Lactams for tuberculosis treatment-:

Tuberculosis infection is resulted due to infection by mycobacterium bacteria which possess β -lactams resistance property. This results in inactivity of β -lactam drugs to treat the tuberculosis infection. Scientists has developed combination therapy of β -lactam drugs along with β -lactamase inhibitor to treat tuberculosis and found effective^{lv}.

To work as an efficient drug against tuberculosis, β -lactam drugs needs to meet the following prerequisites,

- The therapeutic concentration at infected tissue must be reached.
- In order to reach the intracellular mycobacteria in the early stages of infection, it should be able to pass through the membrane of monocyte cells.

For it to exercise its inhibitory impact on the target proteins, specifically PBPs, it must be able to get through the cell walls of monocytes and withstand destruction by baterial enzymes.

The highest susceptibility rate was 74% for the cefazolin–clavulanate combination, 72% for meropenem–clavulanate, 68% for cefuroxime clavulanate, and 62% for amoxicillin–clavulanate.

6.0 β -Lactams- a wide spectrum of activity and Enhanced potency -:

Newer β -lactam/ β -lactamase inhibitor appears much effective and offers improved pharmacokinetic profiles, thus are much effective in the treatment with quite a lesser dose. The less dosage of novel antibiotic/lactamase inhibitor is effective due to its increased antibiotic activity and its resistance towards hydrolysis by β -lactamase as inhibitor acts as “Suicide Killer “for bacteria.

Due to high reactivity, β -lactam antibiotics are being widely prescribed for versatile medicinal applications. However pathogens combat their activity by degradation of antibiotic molecule. Novel β -lactams are developed with wide spectrum of activity in order to persist its activity against bacterial infections

Initially Penicillins were used to treat the infections caused due to gram-positive and gram-negative infections. But on repeated use of pencillin, it became inactive due to the resistance developed by bacterial species, pencillins to treat these type of infections. Henceforth medicinal scientists developed different types of advanced β -lactam compounds with its wide spectrum of antibacterial /antiviral activities.

Oxacillin IV and Nafcillin IV are examples of modern β -lactam antibiotics that were developed to treat bacterial infections caused by gram-positive bacteria. Usually used to treat infections of the skin and soft tissues, these antibiotics target methicillin-susceptible staphylococci (MSSA).

Certain gram-positive and gram-negative infections are treated with natural penicillins, penicillin G (IV) and penicillin V (PO).

Infections caused by both gram-positive and gram-negative bacteria are treated with aminocillins in addition to β -lactamase inhibitors. Amoxicillin (PO) and ampicillin (PO/IV) are used to treat the following types of infections under this category:

- Upper respiratory tract infections (pharyngitis, otitis media, and sinusitis) outbreaks of *Enterococcus faecalis*.
- Infections with listeria.
- Infections within the abdomen

Gram-negative bacteria resistant to amino penicillins (*Pseudomonas aeruginosa*) seem to be susceptible to these β -lactam antibiotics, also referred to as ureidopenicillins. They are also used in combination with β -lactamase inhibitors. Piperacillin and cephalosporins of I, II, and III generations are members of this category. One subclass of cephalosporins is as follows: Cefazolin IV, Cephalexin PO, and Cefadroxil PO are the three main cephalosporins.

Second-generation cephalosporins include cefaclor (PO), cefprozil (PO), cefuroxime (IV/PO), cefoxitin (IV), and cefotetan (IV).

Cefotaxime (IV), ceftriaxone (IV), cefpodoxime (PO), cefixime (PO), cefdinir (PO), cefditoren (PO), and cefibuten (PO) are examples of these third-generation cephalosporins.

Anti-pseudomonal Fourth and sixth generation cephalosporins are included in this group.

Fourth-generation cephalosporins include Ceftazidime (IV), Ceftazidime/avibactam (IV), and Cefepime (IV). Generation Five CEFTOlozone/tazobactam (IV), ceftobiprole (IV), and ceftaroline (IV) are cephalosporins. With their enhanced potency and wide range of action, these novel β -lactams aim to treat a number of illnesses caused by common bacteria such as *Pseudomonas aeruginosa*.

7.0 β -Lactamase Inhibitors:-

The medicinal activity of many β -lactam antibiotics against the bacterial infections is not found to perform to the expected level due to drug resistance developed by bacteria after its repeated use for the treatment of titled disease. This is due to the deactivation of β -lactam antibiotics caused by bacteria by its hydrolysis and this happens to many types of β -lactams like cephalosporins, Penicillin, monobactams and carbapenems. Due to this drug resistance developed by bacteria around 23000 per every year deaths of human being are caused in US itself resulting heavy economic loss of 35 billion dollar^{xxii}

The combination therapy of β -lactams with β -lactamase inhibitor is found effective to treat bacterial infections and ruling out the drug resistant by bacterias.

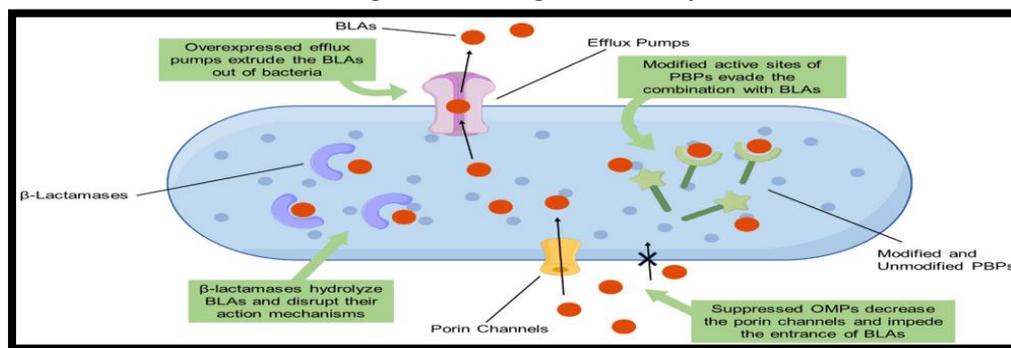


Figure-12: Proposed mechanism of bacterial resistance to beta lactam antibiotics

Beta Lactam antibiotics (BLA) and beta lactamase modifies the active sites of Pencilin binding proteins (PBPs) & regulates outer membrane proteins (OMPs)^{xxvii}. The major threat of β -lactam antibiotics lactam degradation by β -lactamase can be overcome by two strategies with respect to its mechanism of action

I) The development of novel beta lactam antibiotics that are prone to resist its hydrolysis by β -lactamase.

II) Discovery of novel β -lactamase inhibitor. However novel BLA development and its subsequent clinical trials are being costly and time consuming.

There is not any kind of antibiotic resistance in bacteria for β -lactamase inhibitor as it is not being antibiotic. The beta-lactam inhibitor attacks the active site of lactamase and inhibit the complex formation between BLA and bacterial enzyme & thereby the activity of β -lactam antibiotics can be restored in combination with β -lactamase inhibitor.

The novel β lactamase inhibitor are divided as follows

A] Synthetic BLI

B] Natural BLI

7.1 Synthetic β -lactamase Inhibitor (BLI)

Phosphonates, monobactams, and dicarboxylate metal chelate ions function as artificial inhibitors of β -lactamase. Simply put, phosphonates are organophosphorous substances with potent biological properties include antiviral, anti-inflammatory, antibacterial, and anti-cancer effects.^{xv} These are preventing acylation processes at serine active sites, which block the class A, C, and D forms of β -lactamase.^{xxvii-xxviii} Phosphonate derivatives are suppressing the class C type of β -lactamase in specific and time dependent way in *Enterobacter cloacae*.

Antibiotics called monobactams are made from pathogens and inhibit the class C β -lactamase. Aztreonam, a medication in this category, is used to treat a variety of bacterial infections.

The next synthetic BLI is derived from the dicarboxylate metal chelate ion category and has the ability to inhibit. With Zn^{2+} , these compounds' carboxylate group creates chelate compounds. Imipenem and dicarboxylate derivatives work together to efficiently inhibit MBL-producing bacteria that express NDM, IMP, and VIM.^{xxvii} MBLs are suppressed by ANT431, a recently created pyridine-2-carboxylic acid. The bacterial periplasms where MBLs are generated are permeable to ANT431.

7.2 Natural β -lactamase Inhibitor (BLI)

Plants are used to extract some possible β -lactamase inhibitors. Methyl cinnamate, an inhibitor obtained from *Ocimum basilicum*, exhibits comparable activity to the synthetic inhibitor Clavulanic acid.^{xvi} Similar to clavulanic acid, methyl cinnamate functions as an inhibitor by focusing on the bacterial active sites Arg254. Another less cytotoxic natural substance, canosic acid, has a variety of applications as an inhibitor, including in food, medicine, and other natural compounds. Scientists have studied this compound's inhibitory effect and found that it binds to NDM-1's allosteric sites, including Phe46, Tyr64, Leu65, Asp66, and Thr94.

In addition, *Enterobacteriaceae*, *Acinetobacter*, and *Pseudomonas* strains that produce the β -lactamase enzyme can be inhibited by BLIs made from naturally occurring fungal products. A thorough investigation of the cytotoxicity and its interactions with β -lactam drugs is important as a precaution and to make them effective BLIs.^{xxviii - xxix}

8.0 Directed mode of Action (β -Lactamase Inhibitor)-:

The recent research work is focused on emergent β -lactam/ β -lactamase inhibitor being precisely targeting definite β -lactamase classes like A, C and D, leading to more specific and operative prohibitions of bacterial infections and other types of diseases^{xxx}.

Examples of β -lactamase inhibitor combinations are cefazolin– clavulanate, meropenem– clavulanate, amoxicillin–clavulanate some novel β -lactam. Out of this cefazolin– clavulanate combination is seeming more effective as compared with others^{xxx-xxxii}.

The novel 1,4-diaryl 2-Azetidinone Combretastin A-4 (CA-4) analogues are exhibiting strong antiproliferative, antiapoptotic, and tubulin polymerization inhibitory properties.^{xxii} Because it is a crucial component of mammalian DNA and RNA, the thymine-based molecular hybrid hetrocycle Thym1,2,3-triazole- β -lactam has strong anticancer properties in vitro. Cholestrol absorption is effectively inhibited by the β -lactam derivative ezetimibe. In vitro, it is discovered that the combination of carbapenems and clavulanate is an effective treatment for pan-resistant strains of M-tuberculosis. However, only clinical trials can determine its ideal dosage schedules and therapeutic efficacy.

The bacterial enzyme causes the hydrolysis of penicillin β -lactam ring due to its strained nature. The figure 14 depicts the schematic representation of hydrolysis step involved according to which lactamase enzyme (Serine 70) nucleophilically attacks the strained β -lactam ring producing acyl enzyme which in turn get cleaved into penicilloic acid and enzyme.

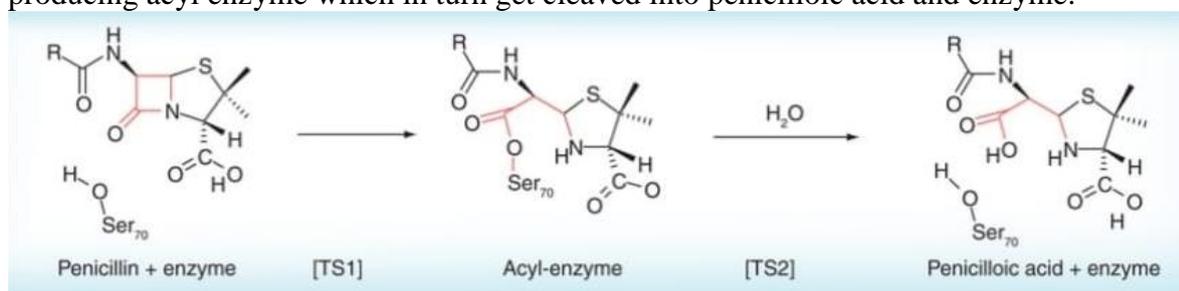


Figure-13: β -lactam (Penicillin) hydrolysis steps by bacterial enzyme

n order to work as potent antibiotics against tuberculosis infection, the effective inhibition of β -lactam is very important to get clinically implemented^{xxxii}. The currently available BLI (β -lactamase inhibitor) acts as substrate for bacterial enzyme (β -lactamase) and acts as a suicide inhibitor which helps to restore the antibacterial activity of β -lactam antibiotics.

The stages involved in the effective inhibitor Clavulanate's suppression of the β -lactam molecule are shown in Figure 14. This method states that the substrate binds and forms the acyl enzyme, which in turn causes the β -lactam to open. The cyclic enol group in the opened β -lactam ring is a good leaving group because it opens the second ring, which creates a reactive imine and subsequently rearranges to generate an enamine. Aldehyde and dehydrated aldehyde are the ultimate products of this enamine's decarboxylation process. When clavulanate binds to the β -lactamase enzyme as a substrate, effective inhibition begins, and the production of Acyl enzyme is continued, eventually forming stable Acyl enzyme upon further rearrangement.^{xxv}

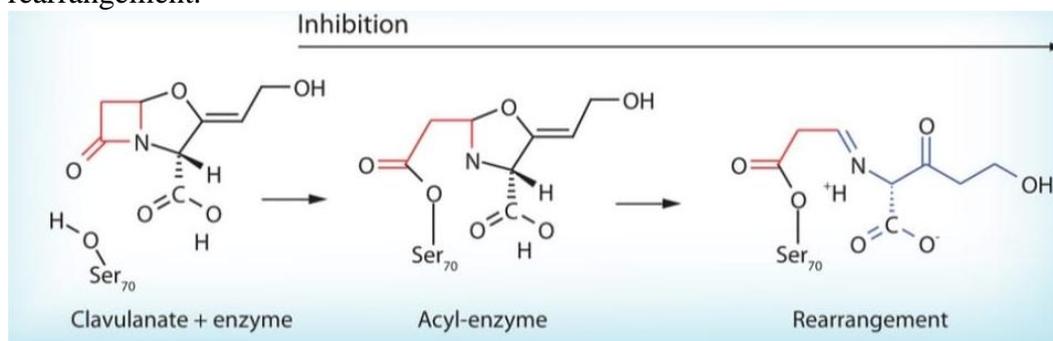


Figure-14: Inhibition of β -lactamase by Clavulanate-Mechanism

8.1.1 Ceftazidime-avibactam:

The combination of ceftazidime with avibactam is used intravenously as antibiotic along with metronidazole to treat bacterial infection observed in abdominal part. It is also working effectively against pneumonia developed in patients who are on ventilators or those are in hospital and kidney and urinary tract infections. In this combination therapy, ceftazidime is from cephalosporin category whereas avibactam is β -lactamase inhibitor. The Ceftazidime is killing bacteria and avibactam prevents its breaking down by bacteria^{lxxi}.

This combination therapy does not work against cold, flu or any other viral infections.

8.1.2 Meropenem-vaborbactam:

Actually Carbapenem is being the strongest class of β -lactam that does not easily hydrolysed by β -lactamase enzyme. However it is not found stable against Enterobacteriaceae which led to the search for new antibacterial. The complicated urinary tract infection including pyelonephritis are treated effectively by the pairing of Meropenem with vaborbactam. It is used by intravenously. This pairing antibiotics combination is being the first one which has got approval for the treatment of complicated Urinary tract infections in USA^{lxxi}.

The potent inhibitor Vaborbactam is acting as a substrate for the class A Serine Carbapenemases (bacterial enzyme) thereby inhibiting the degradation of meropenem and in turn restoring its antibiotic activity against β -lactamase producing Enterobacteriaceae^{xxxiii}

8.1.3 Imipenem-relebactam:

There are some alternative carbapenem based combinations with some innovative β -lactam/ β -lactamase which are very effective against the broader resistant bacteria. The novel combination Imipenem-Cilastatin -relebactam^{lxxi} is very effective for the treatment of complicated urinary tract infections. Relebactam is being potent inhibitor having ability to inhibit the broad spectrum of β -lactamases like class A & C including carbapenemases. The relebactam avoids the degradation of Imipenem by bacterial enzymes and restores the antibacterial activity of Imipenem^{xxxiii}

8.1.4 Ceftolozane-tazobactam:

Another group that are mostly operative against Gram negative bacteria, counting certain resistant strains. The enhanced antipseudomonal activity is obtained by pairing combination of Ceftolozane-tazobactam (TOL-TAZ). Here β -lactam antibiotic Ceftolozane^{lxxi-lxxii} (a new antipseudomonal cephalosporin) combines with Tazobactam (a classic β -lactamase inhibitor). This pairing combination is used to treat urinary tract infections and abdominal infections. It can also be used certain types of pneumonia which are developed in hospitalized people or those are on ventilators.

9.0 Challenges and future direction of research

Medicinal practitioners are prescribing β -lactam antibiotics preferably due to its safety and well tolerance as compared to other types of antibiotics. However it can have some side effects like allergic reactions. Apart from allergic reactions, there are other types of side effects induced by use of β -lactam antibiotics. Some of these are as below,

- Impaired hemostasis due to defective platelet aggregation is caused due to use of Penicillin and Piperacillin.
- Cardio respiratory arrest & death can be resulted due to use of Benzathine intravenously.
- At rare instances bone marrow depressions are caused by use of cephalosporins.
- Some of the Cephalosporins are nephrotoxic and are associated with necrosis. Due to the displacement of bilirubin by Albumin in neonates, jaundice can be caused by Ceftriaxone.
- Biliary pseudolithiasis is caused due to its high affinity for biliary calcium.
- High doses of Imipenem to patients suffering from CNS lesions or renal insufficiency.

The side effects of β -lactam antibiotics and its inactivity due to bacterial enzymes are being the challenges associated while developing novel β -lactam drugs. The innovator needs to take care

of these challenges while doing research on novel β -lactam molecules. Medicinal chemist /Scientist should consider these aspects to increase the stability towards bacterial enzymes either incorporating the proper substituent or adopting the novel chemistry that will enhance the antibiotic activity. By use of advanced technology such as artificial intelligence, advanced biotechnology, organ-on-chip technology, Virtual clinical trials can lead the development of new molecules with desired characteristics..No doubt these activities are time consuming and costly.

10.0 The future of β -lactams as a sensors

The penicillin containing β -lactam ring is interpreting the effectiveness of antibiotics by inhibiting cell wall synthesis during bacterial replication. During the cleavage of β -lactam, proton is being released which results in acidic side pH. A sensor is a device that responds to physical stimuli & transmits the observed impulse for measurement.

The biosensors are used to observe the enzymatically glucose levels using oxygen electrodes to observe the products of glucose oxidase. The work of sensors is accomplished by using a recognition of element and transducer. The recognition element responds to the changes occurring in the environment and transducer converts these changes into electronic signals which helps to observe the surrounding changes. The biosensors are an analytical device that monitors and transmits the changes observed. The biosensors involves the biological component like enzymes or bacteria which reacts with the target moiety that gives signal for analysis. Recently new sensing system namely pseudo sensor has been developed to measure the action of enzymes such as the activity of beta-lactamase. The different bio sensing systems like molecular, cellular and tissue based are available. Cell based bio sensing are more effective as compared with other bio sensing system due to its stability towards varying temperature and pH which makes them physiologically stable and allowing the conduction of bio-availability studies^{xxvii}. In this bio sensing system, transducer is replaced by fluorescent proteins like green fluorescent proteins (GFP),aequorin,firefly luciferase, bacterial luciferase and red fluorescent proteins

This research study can be extended to the study of enzyme functioning in human body which are present in the structure involving β -lactam ring. The different compounds can be used as sensing agents to treat the patients or to give them certain drugs for early recovery.

All these sensing devices were developed to find the ways for the deactivation of β -lactamase bacterial enzyme to get the continued β -lactam antibiotics usage without developing drug resistance in living cells^{xxvii, xxix}. These sensing devices will be used to detect, measure and treatment of patient suffering by diabetes and cancer

11.0 Grouping Therapy:-

Discovering permutations and combinations of β -lactam-based antibiotics with new antibiotic classes to hold an auxiliary enriched ability against multidrug-resistant organisms.

Grouping therapy is nothing but a combination therapy. Basically, β -lactams are very effective against bacterial infections, and their contribution, as per the doctor's prescription, is around 65% among other antibacterial antibiotics. The expenditure on these antibiotics is also around USD 15.

However, due to the development of mutagenic enzymes by bacteria or pathogens, these antibiotics can cause degradation or hydrolysis, rendering them inactive in the treatment of infections. Therefore, individual antibiotics will not be effective after its repeated use for curing the infected disease. In such cases, grouping therapy is the best option for its treatment whereby one antibiotic antibioticsed either with /or another antibiotic and enzyme inhibitor. Here, the Inhibitor acts as a suicide killer and restores the activity of the antibiotic. e.g., Ceftazidime-avibactam, Ceftolozane-tazobactam: cefazolin, clavulanate etc. This overcomes the multidrug-resistant problem associated due to β -lactamase^{xxvi-xxvii, xxix}.

Novel cancer drugs can be designed and developed by using new concept of medicinal chemistry known as molecular hybridization. This phenomenon can be explained on the basis of pharmacophoric moieties combination with different bioassay to synthesize novel hybrid compounds with enhanced with bioassay as compared to parent drugs. Research strategies like this can be used for the synthesis of novel compounds with modified selectivity, also different strategy for dual modes of action and also to reduce harmful side effects of antibiotics.

Conclusion

Medicinal Chemistry is playing vital role in the lives of living organism and β -lactam serves as moiety in variety of antibiotics. It was basically developed for antibacterial disease treatment, but the medicinal scientists have developed different methodology to prepare different β -lactam compounds to treat different types of disorder/diseases viz anti-inflammatory, analgesics drugs, anticancer drugs, cholesterol absorption inhibitor, anti-diabetic drugs etc. Molecular hybridization is an advanced technique for synthesis of novel anticancer drugs which involves the combination of two bio active molecules. Due to reactivity of β -lactam Scaffolds, these antibiotics are efficacious & there is wide futuristic scope for development of new medicinal moieties containing β -lactam scaffolds. β -lactamase inhibitor is also efficacious concept for novel antibiotics development in drug chemistry. The application of β -lactam compounds as a biosensors is also one of the great invention to detect the life threatening diseases like cancer and diabetes at its initial level ultimately resulting in its immediate curative practices. There is continuously wide scope for further research and development of novel β -lactams moieties due to its specific chemical scaffold and effective biological activity.

LIST OF ABBREVIATIONS

PGN- Peptiglycogen
PBP-Pencillin Binding Proteins
SAR-Structure activity relation
GLT-Glucamate
DM-Diabetes Mellitus
BLI-Beta lactam Inhibitor
BLA-Beta Lactam antibiotics
CUTI-Complicated Urinary Tract Infections
BLM-Beta Lactam
CFX-Ceftriaxone
CA-Combretastatin
LPS-lipopolysaccharides
MH-Molecular Hybridisation

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