AZETIDINONES (BETA-LACTAMS)

The β-lactams are 4-membered cyclic amides derived from 3-aminopropanoic acids. Though the first member synthesized by Staudinger in 1907, the β-lactams as a class acquired importance since the discovery of penicillin which contains β-lactam unit as an essential structural feature of its molecule, this interest continued unabated because of the therapeutic importance of β-lactam antibiotics and recent finding of new naturally occurring β-lactams. As a result of vigorous research, a vast literature has been accumulated over the years, and the chemistry of azetidinones continues to be blossoming field.

Recent years have seen a resurgence of interest in the development of stereo and enatioselective methodologies. The utility of azetidinones as synths for various biologically active compounds, as well as their recognition as cholesterol absorption inhibitors and enzyme inhibitors has given impetus to these studies.

The β-lactams are 4-membered cyclic amides derived from 3-aminopropanoic acids. Though the first member synthesized by Staudinger in 1907, the β-lactams as a class acquired importance since the discovery of penicillin which contains β-lactam unit as an essential structural feature of its molecule. In the late 1990s, several groups reported novel methodologies for the synthesis of azetidinones of potential biological activities by applying known methods.

2.1. Chemistry of Azetidinones

In the literature, monocyclic β-lactams are usually referred to as azetidin-2-ones or 2-oxoazetidine, based on the nomenclature of the parent heterocycle, azetidine. However, the trivial names penam for the fused β-lactam (201 a) and cepham for the bicyclic system (202 a) are also used. Similarly, the term o-openam, o-cepham, azapenam and azacepham were coined for the bicyclic β-lactam (201 b), (202 b), (201 c) and (202 c) respectively. This trivial system of nomenclature is inadequate, especially in the case of fused β-lactams having no bridge head nitrogen atom, and in those having no heteroatom at position 1 or alterations in the positions of the hetero atom of the non β-lactam ring. This discrepancy can be removed by adopting a new system in which fused β-lactams (203) and (204) may be called “Alkanam” and “isoalkanam” respectively. Thus, β-lactams containing 7, 8 and 9
atoms in the bicyclic system (203) may be given generic names, heptanam, octanam, nonanam and so on, using the corresponding latin roots. The numbering system as shown in (201 d) and (202 d) is in conformity with the convention followed in the case of penam-cepham nomenclature. Thus, the conventional penam will be termed as 1-thiaheptanam, and cepham as 1-thiaoctanam according to this system. Similarly, the fused β-lactams of the type (204) may be termed as isoheptanam, isoctanam, isononanam and so on, depending on the number of atoms in the bicyclic system. The numbering of ring atoms in this case may be the one used for azetidin-2-ones, and is shown in (205).

(a) $Z = S$, (b) $Z = O$, (c) $Z = NH$, (d) $Z = CH_2$

A bicyclic β-lactam containing a double bond in the ring system may be given the corresponding generic name derived from the collective name “Alkenam” or “Isoalkenam” depending on the mode of fusion of the rings. For stereo description of the molecule, the terms “α” and “β” denoting the configuration of the substituents, which may be below or above the plane of the β-lactam ring, may be used as in case of steroids.

**Construction of β-lactam ring**

There are diverse synthetic routes to β-lactams\textsuperscript{151-155} and in principle the 4-membered heterocycle could be constructed by the formation of one, two, three or all four bonds of the ring system during the process of cyclisation\textsuperscript{156-171}.
**Cyclisation of 3-aminopropanoic acid derivatives**

Five 1,3,4-triarylazetidin-2-ones (207) were prepared by treating (206) with benzenesulfonyl chloride and alkali. Saponification of 2, 2-disubstituted-3-benzamido propanoic acid esters (208) was found to give β-lactams besides the acid derivatives\(^\text{172}\). The cyclisation is possibly initiated by removal of the amidic proton, followed by Dieckmann reaction (Scheme 43).

\[
\begin{align*}
R_2 & \quad R_3 \cdot C-CH(R_4)\cdot COOH \\
\text{NH} & \quad R_4 \\
R_3 = H, \ R_1 = R_2 = & \quad \text{Aryl} \\
\end{align*}
\]

\(\text{(206)}\)

\[
\begin{align*}
\text{Ph} & \quad \text{Ph} \\
\text{PhCO} & \quad \text{NH} \\
\text{COO} & \quad \text{Bu-1} \\
\end{align*}
\]

\(\text{Saponification} \quad \text{Acidification}
\]

\[
\begin{align*}
\text{Ph} & \quad \text{Ph} \\
\text{PhCO} & \quad \text{N} \\
\end{align*}
\]

\(\text{(208)} \quad \text{PhCO} \quad \text{N} \quad \text{(209)}\)

(Scheme 43)

Bicyclic β-lactams such as penicillins\(^\text{173,174}\), cephalosporin\(^\text{175}\) analogs\(^\text{176,177}\) and the compound (210)\(^\text{178}\) were synthesized by this method, using carbodimides as Cyclising agents.

\[
\begin{align*}
\text{Ph} & \quad \text{Ph} \\
\end{align*}
\]

\(\text{(210)}\)

Blinkovsky et al\(^\text{179}\) have suggested the synthesis of beta-lactam antibiotics containing alpha-aminophenylacetyl group in the acyl moiety catalyzed by D-phenylglycyl-beta-lactamide amidohydrolase.

42
Carlos Cativiela et al\cite{18} have reported the asymmetric Synthesis of Beta-lactams by Diastereoselective Alkylation of Chiral 2-Cyano Esters.

**Addition of imines**

The first \(\beta\)-lactam was prepared by the ketene-imine interaction. Usually ketenes are generated *in situ* by dehydrohalogenation of suitable acetyl chlorides in the presence of a tertiary base. Also, photolysis and thermal decomposition of diazoketones were employed for generating ketenes, which were trapped by imines to give \(\beta\)-lactams. Thermal fragmentation of acetylenic ethers to aldoketenes was also reported.

The choice of ketene precursor is important, because it gives \(\beta\)-lactams with a suitable group at the carbon atom \(\alpha\) to the \(\beta\)-lactam carbonyl function\cite{154}. The structural requirements of the imines are difficult to define due to the inconsistency in the results obtained from different procedures. Imidylchlorides, \(o\)-alkyloximes and phenylhydrazones did not give azetidin-2-ones. Addition of diphenyl ketenes on acyl hydrazones is reported to give \(\beta\)-lactams. Imines such as (210 a) and (210 b) gave (211 a) and (211 b), on treatment with diphenyl- and dimethylketenes respectively\cite{153}. Tert-butylycyanoketenes with imino ethers gave \(\beta\)-lactam (211 c)\cite{181}. Diphenyl ketenes with imines (210 d) gave \(\beta\)-lactams (211 d), but their reactivity and yields varied considerably with change in the substituents in the aromatic ring. Conjugated diimines\cite{182,183} and carbodiimides\cite{154} also gave \(\beta\)-lactams with suitable ketenes (**Scheme 44**).

\[
\begin{align*}
R_1 - C &= N - R_3 \\
R_2
\end{align*}
\]

(a) \(R_1 = \text{morpholine, } R_2 = H, R_3 = \text{Ph, } R_4 = R_5 = \text{Me or Ph}\)

(b) \(R_1 = \text{Ph, } R_2 = \text{MeS}, R_3 = \text{Ph, } R_4 = R_5 = \text{Me or Ph}\)

(c) \(R_1 = H, R_2 = -\text{OMe, } R_3 = R_4 = \text{t-Bu, } R_5 = \text{CN}\)

(d) \(R_1 = R_3 = \text{Substitutedphenyl, } R_2 = H, R_4 = R_5 = \text{Ph}\)

**Scheme 44**
Reactions of isocyanates

Diazomethane was found to give β-lactams (213) when treated with phenyl- and p-bromophenylisocyanates\textsuperscript{184}. Indolyl-3-isocyanate reacted similarly\textsuperscript{152}.

![Reaction of Diazomethane](image)

(Scheme 45)

Ring expansion of 3-membered rings

The aziridine (214) in the presence of thionylchloride or oxalylchloride rearranges to β-lactam (215) in benzene, possibly via a mixed anhydride which undergoes ring expansion. The conversion is stereospecific and yields are good\textsuperscript{185} (Scheme 46).

![Ring Expansion Reaction](image)

(Scheme 46)

Ring contraction of 5-membered rings

Photolytic Wolf rearrangement of 3-diazopyrrolidin-2,4-diones (216), in the presence of tert-butylcarbazate afforded β-lactams (217)\textsuperscript{186,187}. This method was extended to the synthesis of azetidin-2-one (218), which was found to be biologically inactive\textsuperscript{188}. The fused system (219) under similar conditions produced (220), which was found to be highly unstable\textsuperscript{189} (Scheme 47).

![Ring Contraction Reaction](image)

(Scheme 47)
Passerini reaction

The reaction of carbonyl compounds with 3-aminopropanoic acids, followed by treatment with a suitable isocyanide afforded β-lactam derivatives. This is an extension of the Passerini reaction and it was useful for the preparation of monocyclic and bicyclic β-lactams (222) and (223) respectively. The reaction envisages formation of a cyclic compound (211) which on transannular acyl migration gave the β-lactam (222). It is noteworthy that the configuration of newly formed asymmetric center in the penicillin analog (223) is predetermined by the steric disposition of the reacting molecule (Scheme 48).
Rearrangement reactions

There were several cycloadducts which undergo thermal or photochemical fragmentation, generating ketenes and imines which recombine to give β-lactams. This method is of limited use because of the drastic conditions involved and possible side reactions. Beckmann rearrangement of o-sulfonyloximes (224) was reported to give novel β-lactams (225) but now the revised structure has been proposed.\textsuperscript{154}

![Cycloadduct](image)

Reactions of Beta-lactams

Cleavage of the β-lactam bond

The β-lactam bond undergoes rupture in the presence of an alkali, acid and β-lactamase, yielding 3-aminopropanoic acids. By selective degradation the natural β-lactams could afford useful amino acids. In the presence of dry hydrogen chloride, a β-amino acid hydrochloride is generated. For example, the compound (226) gave (227) on treatment with hydrogen chloride in methylene chloride.\textsuperscript{190} Similarly, the β-lactam may be cleaved by imines\textsuperscript{191} (Scheme 49).

![Cleavage](image)

Cleavage of the 2,3-bond in azetidin-2-ones

1-Haloazetidin-2-ones (228) undergo photolytic or thermolytic cleavage to give isocyanates (229) capable of undergoing secondary cyclisation under suitable conditions.\textsuperscript{192} Similarly, 3-azidoazetidin-2-one (230) on refluxing in diglyme, underwent ring expansion through 2,3-bond cleavage (Scheme 50).
Cleavage of 5,6-bond in penicillin

Rearrangement of penicillin to penilloic acid (233) involves cleavage of the 5,6-bond\textsuperscript{186} (Scheme 51). Similar bond cleavage was observed in penicillin-1-oxide\textsuperscript{195}.

Cleavage of the 1,4-bond in azetidin-2-ones and collapse of the bridge in bicyclic \( \beta \)-lactams

\( \beta \)-Lactams bearing a C-4 hetero atom are unstable and easily undergo 1,4-bond cleavage\textsuperscript{196}. For example, the 4-mercaptoazetidin-2-one (234) changes to isothiazolinone (235) in 40% yield, on treatment with dimethylsulfoxide\textsuperscript{197} (Scheme 52).
**Fragmentation of Beta-lactams**

Monocyclic Beta-lactams on photolysis or thermolysis break up into ketenes and imines or alkenes and isocyanates, depending on the substituents present in the molecule and which ever fragmentation is energetically profitable\(^ {198} \). This process is essentially a case of retrocycloaddition. Reagent induced fragmentation leads to diverse products, depending on the substituents and reagents used. Fragmentation of penicillin\(^ {199} \) and cephalosporin\(^ {200} \) occurred on treatment with trifluoroacetic acid, the fragments being amido ketenes, and \( \Delta^2 \)-thiazoline and \( \Delta^2 \)-1,3-thiazine derivatives respectively. Sometimes the fragment formed as primary products may undergo secondary reactions. For example, \( \beta \)-lactam (236) on retro Michael reaction, gave (237) and subsequently (238) and (239)\(^ {201} \) (Scheme 53).

\[
\begin{align*}
\text{Ph} & \quad \text{O} \\
\text{S-CH}_2\text{CH}_2\text{CO}_2\text{Me} & \quad \xrightarrow[]{} \\
\text{N} & \quad \text{Ph} \\
\text{CO}_2\text{Me} & \\
\text{Ph} & \quad \text{N} \\
\text{S} & \\
\text{Ph} & \quad \text{Ph} \\
\text{N} & \\
\text{CO}_2\text{Me} & \quad \xrightarrow[]{-\text{PhC}=\text{C}=\text{O}} \\
\text{S} & \\
\text{Ph} & \\
\text{O} & \\
\end{align*}
\]

(Scheme 53)

Enzyme catalyzed fragmentation of benzylpenicillin was reported\(^ {202,203} \). It is noteworthy that the azido group in \( \beta \)-lactam (240 a) on reduction with Adam’s catalyst and subsequent-acylation with phenoxyacetylchloride and triethylamine afforded the 6-phenoxy compound (240 c)\(^ {204} \). Such an unusual result may be explained only on the assumption that the 6-amino compound (240 b) undergoes fragmentation and generates a \( \Delta^2 \)-thiazoline, which then reacts with phenoxyacetyl chloride and triethylamine in the usual way.

\[
\begin{align*}
\text{Ph} & \quad \text{S} \\
\text{Z} & \quad \text{R} \\
\text{O} & \\
\text{R} & \quad \text{H, Me} \\
\text{Z} & \quad \text{N} \text{Z}, \ (b) \text{Z} = \text{NH}_2, \ (c) \text{Z} = \text{PhO} \\
\end{align*}
\]

(240)
2.2. Biological Importance of Beta-lactam Derivatives.

2.2.1. Beta-lactam derivatives as antimicrobial agents.

Azetidine and their derivatives have been extensively explored for their applications in the field of medicine\textsuperscript{205-209}. Likewise, azetidin-2-ones are of great importance because of \(\beta\)-lactam derivatives as an antibacterial agent\textsuperscript{210-214}. Recently, incorporation of these compounds have witnessed a great upsurge in the treatment of tuberculosis and other chemotherapeutic diseases\textsuperscript{215}. Sharma \textit{et al}\textsuperscript{216} reported synthesis and antibacterial activity of some N-sulphonamoylphenylamino-3-chloro-4-phenylazetidin-2-ones. Most of the compounds exhibited significant antibacterial activity. Comp.1 [4(5,6 dimethoxy pyrimidino sulphonamoyl)phenylamino]-3-chloro-4-phenylazetidin-2-one (241) has been found to be very potent compound against \textit{E. coli}.

\[
\text{RNHSO}_2\text{NH} - \text{N} - \text{Cl} \\
\text{R} = 4,5\text{-dimethoxypyrimidyl}
\]

(241)

A series of 1-[5-(N\textsuperscript{10}-phenothiazinomethyl)-1,3,4-thiadiazol-2'-yl]-4-substituted-2-azetidinones as antifungal agents have been reported by Rawat \textit{et al}\textsuperscript{217}. All the compounds were screened for their antifungal activity against the fungi \textit{Candida albicans}, \textit{Rhizopus oryzae} and \textit{Crysosporium pannical}\textsuperscript{218}. The fungicidal data indicated that all the compounds were moderately to highly toxic. The toxicity of compounds depends upon the nature and position of the substituents at the aryl moiety. Compound (242) displayed promising antifungal activity.

Shah \textit{et al}\textsuperscript{219} synthesized azetidinones (243) from hydrazine thieno [3,2-d]pyrimidines as potential antimicrobial agents. All the products have been evaluated for their \textit{in vitro} growth inhibitory activity against several microbes like \textit{B. megatilis},
*B. subtilis, E. coli, A. aerogens* and *A. awamori*. Most of the compounds exhibited maximum activity in the range of 21-27 mm against *A. aerogens*. Other compounds showed either moderate or less activity against these organisms. None of the compounds synthesized was found to exhibit significant activity against *B. subtilis*.

![Chemical structure](image)

\[ R = \text{aryl/substituted aryl group} \]

(243)

Parmar *et al*\textsuperscript{220} reported synthesis of azetidinones from hydrazinopyrimidine as potential antimicrobial agents. All the products were evaluated for their *in vitro* growth inhibitory activity against several microbes like *B. megaterium, B. subtilis, E. coli, P. fluorescens* and *A. awamori*. All the compounds exhibited mild to moderate antimicrobial activity against all microorganisms except (244) which exhibited promising activity with ampicillin and chloramphenicol against *P. fluorescens*.

![Chemical structure](image)

\[ R = \text{aryl/substituted aryl group} \]

(244)

Antimicrobial activity of azetidin-2-ones has also been reported by various authors\textsuperscript{221-228}.
Mechanism of action of Beta-lactam derivatives as Antimicrobial agents.

Beta-lactams inhibit cell wall synthesis (Figure No. 1). The peptidoglycan is composed of sugars and amino acids. The sugar components consist of alternating residues of N-acetyl glucosamine and N-acetyl muramic acid residues. Peptide chain of 3-5 amino acids is attached with N-acetyl muramic acid. The peptide chain can be cross linked to peptide chain of another strand forming mesh like layer by transpeptidase. Beta-Lactams bind to PBPs which catalyse transpeptidation reaction. They inhibit transpeptidation (final stage in the synthesis of cell wall) (Figure No. 2).
2.2.2. **Beta-lactam derivatives as antitubercular agents.**

Synthesis and antitubercular activity of Beta-lactam derivatives\(^{229-232}\), has been reported by different authors. The representative compounds were tested *in vitro* for their anti-tubercular activity against *M. tuberculosis H37Rv*. The data were compared with standard drug Rifampin. All the compounds showed moderate antitubercular activity against *M. tuberculosis*.

Patel *et al*\(^{233}\) have reported synthesis and antitubercular activity of 2-[4-(4-substitutedphenyl)-3-chloro-2-azetidinon-1-yl]-4-[2-(4-chlorobenzene sulphonamido)-phenyl] thiazoles (245). Primary screening of the compounds for antitubercular activity was conducted at 12.5 mcg/ml against *M. tuberculosis H37Rv*. Compounds demonstrating at least 99% inhibition in the primary screening were tested at lower concentrations against this microorganism to determine actual minimum inhibitory concentration. The antitubercular activity data showed that most of the azetidinone derivatives exhibited 100% inhibition in the primary screen at 12.5 mcg/ml concentration.
Vashi et al\textsuperscript{234} have reported synthesis and antitubercular activity of 2-azetidinones bearing thymol moiety. The products displayed moderate to good tuberculostatic activity. Synthesis and antitubercular activity of 2-(4-aryl-3-chloro-2-azetidinon-1-yl-amino)-6-(4-chlorophenyl)-5-cyano-3-N-methyl-3,4dihydropyrimidin-4-ones is reported by Modha et al\textsuperscript{235}. All the products displayed mild to moderate antitubercular activity against \textit{M. tuberculosis}. Compound (246) was the most active member of this series.

\textbf{2.2.3. Beta-lactam derivatives as anti-inflammatory agents.}

Several such comp. like 1-[5-(carbazolylmethyl)-1,3,4-thiadiazol-2-yl]-4-(substituted phenyl)-3-chloro-2-oxo-azetidines have been synthesized and evaluated for their anti-inflammatory activity by Srivastava \textit{et al}\textsuperscript{236}. All the compounds displayed mild to moderate anti-inflammatory activity except compound (247) that showed anti-inflammatory activity that was comparable to standard drug phenylbutazone.
Several comp. like 1-[5-(N\textsuperscript{10}-2-chlorophenothiazinomethyl)-1,3,4-thiadiazol-2-yl]-4-(substituted phenyl)-3-chloro-2-oxoazetidines have been synthesized and evaluated for their anti-inflammatory activity by Srivastava et al\textsuperscript{237}. All the compounds tested for anti-inflammatory activity exhibited mild to moderate activity. The compound (248) was the most potent and active member of this series. It displayed comparable anti-inflammatory activity but lesser than the standard phenylbutazone.

\[
\text{(248)}
\]

2.2.4. Beta-lactam derivatives as anticancer agents.

Shah. et al\textsuperscript{238} thoroughly analysed the mechanism of inhibiton of human leukocyte elastase (HLE) by a monocyclic lactam. This work led to the identification of 4-[(4-carboxyphenyl)-oxy]-3,3-diethyl-1-[(phenylmethyl) amino] carbonyl] -2-azetidinone as the first orally active inhibitor of human leucocyte elastase (HLE). Analogue with different substituents on the urea-N were synthesized and evaluated for their activity in vitro against HLE as well as in vivo in a hamster lung haemorrhage model. Compounds with a methyl or methoxy group in the para position of the benzene ring were very potent in both assays. Park et al\textsuperscript{239} synthesized and evaluated two known phenolic metabolites of paclitaxel. The C3-phenolic metabolite of paclitaxel was prepared from 7-(triethylsilyl)-baccatin III and enantioenriched N-benzoyl-2-azetidinone. The C2 – phenolic metabolite was synthesized from paclitaxel via selective C2 debenzoylation reacylation. Both the metabolites were found to have good anticancer activity. Spletstoser et al\textsuperscript{240} synthesized and evaluated a novel paclitaxel derivatives. The synthesis involved the preparation of an azide-containing C-13 side chain through a Staudinger cycloaddition followed by a growth and a variety of other cell lines. Compounds inhibited tubulin polymerization with potencies commensurate with their cytotoxic activity and a more lipase-mediated kinetic resolution through which azetidinone in 99% cc was obtained. Coupling of the
enantiopure side chain precursor to 7-TES-baccatin and subsequent silyl ether deprotection afforded 3’-(4-azidophenyl)-3’-dephenyl paclitaxel, which was shown to be as active as paclitaxel in tubulin assembly and cytotoxicity assays. A series of novel 1,4-diaryl-2-azetidinones was synthesized by Sun et al\textsuperscript{241} using stereo specific staudinger reaction as conformationally restricted analogues of combrestatin because molecular modeling studies suggested close geometric similarities. They were evaluated for cytotoxicity against a number of human tumor and normal cell lines. Strong potencies were observed, with the best compound exhibiting IC (50’s) of 25-74 nm against human neuroblastoma IMR 32 cell soluble aniline-containing analogue was found to be very effective in inhibiting the growth of AR42J rat pancreatic tumors when transplanted into the nude mice. Boge et al\textsuperscript{242} synthesized and evaluated novel cyclohexyl analogues of taxol and taxotere. Compound 2-(cyclohexyl carbonyl)-2-debenzyolbaccatin was prepared from baccatin by hydrogenation. Subsequent coupling of 2-(cyclohexyl carbonyl)-2-debenzyolbaccatin with N-t-BOC-3-[(tert-butyldimethylsilyl)oxy]-4-phenyl-2-azetidinone, followed by removal of the protecting groups afforded 2-(cyclohexyl carbonyl)-2-debenzoyl taxotere. In a similar synthetic sequence, 3’-cyclohexyl-3’-dephenyl taxol was prepared from N-benzoyl-3-[(tert-butyldimethylsilyl)oxy]-4-cyclohexyl-2-azetidinone and (triethylsilyl) baccatin. The taxol analogue, in which all three taxol phenyl groups were substituted by a cyclohexyl moiety, was synthesized in one step from taxol via hydrogenation. All analogues exhibited strong activity in the microtubule assembly assay and cytotoxicity comparable to taxol against B16 melanoma cells. Different authors\textsuperscript{243-251} have reported the Synthesis of anticancer Beta-lactams & their mechanism of action.

2.2.5. Beta-lactam derivatives as cholesterol lowering agents.

Fluorescent analogues of the cholesterol absorption inhibitor (CAI), have been synthesized by Burnett et al\textsuperscript{252} as enantiomers. Biological testing revealed that they were potent cholesterol absorption inhibitors (CAI’s) and were suitable tools for the investigation of the azetidinone cholesterol absorption inhibiting mechanism of action. Ezetimibe\textsuperscript{253}, (1-(4-fluorophenyl) -(3R) -[3-(4-fluorophenyl) -(3S) hydroxyl-propyl] - (4S)-(4-hydroxyphenyl)-2-azetidinone potentially and selectively inhibited the intestinal absorption of cholesterol, thereby reducing plasma cholesterol in
preclinical models of hypercholesterolemia. In rhesus monkeys fed a diet containing 375mg/day of cholesterol, 0.1mg/kg of ezetimibe completely prevented the doubling of plasma cholesterol normally induced under these dietary conditions (ED$_{50}$=0.0005mg/kg). Low-density lipoprotein (LDL) cholesterol was dose dependently reduced, while high-density lipoprotein (HDL) cholesterol and plasma triglyceride were unchanged. Clader et al$^{254}$ synthesized a series of azetidinone cholesterol absorption inhibitors (CAI) and compounds were evaluated for their activity to inhibit hepatic cholesteryl ester formation in a cholesterol fed hamster model. Although originally designed as acyl CoA: cholesteryl acyl transferase (ACAT) inhibitors, comparison of in vivo potency with in vitro activity in a microsomal ACAT assay indicated no correlation between activity in these two models. Examination of the in vivo activity of a range of compounds has revealed clear structure-activity relationships consistent with a well defined molecular target. Two derivatives, of a novel cholesterol absorption inhibitors were glucuronidated with the help of glucuronyl transferase derived from bovine and dog liver microsomes. An efficient procedure for the iodination was developed on an analytical scale to be used for the preparation of a radioactive$^{255}$ glucuronide. Different authors$^{256-258}$ have reported the Beta-lactam derivatives as cholesterol lowering agents.

2.2.6. Beta-lactam derivatives as human tryptase & chymase inhibitors.

Sutton et al$^{259}$ prepared a series of non guanidine N1-activated C4-carboxy azetidinone tryptase inhibitors by solid phase methodology to quickly assess the SAR associated with the distal functionality on the N1-activating group. From these studies, potent inhibitors with improved specificity were discovered. Qian et al$^{260}$ synthesized a highly stereo selective novel tryptase inhibitor. Key to this synthesis was the discovery and development of a high diastereo selective demethoxy carbonylation of diester to form the trans-azetidinone. Derivatives of 3-benzylazetidine-2-one were designed and evaluated as a novel series of chymase inhibitors by Aoyama et al$^{261}$. Structure activity relationship of 3-benzylazetidine-2-ones led to compounds, which exhibited 3.1nm inhibition of human chymase and enhancement of stability in human plasma (t$_{1/2}$=6hrs). Bisacchi et al$^{262}$ synthesized a number of potent azetidinone tryptase inhibitors in which the guanidine moiety at the
ring C-3 position is replaced with primary or secondary amine or amino pyridine functionality. These compounds were found to be highly potent tryptase inhibitors, which has excellent selectivity against trypsin and most other related serine proteases. Different authors\textsuperscript{263,264} have reported the Beta-lactam derivatives as human tryptase & chymase inhibitors.

2.2.7. Beta-lactam derivatives as anti-hepatitis agents.

Hepatitis A virus (HAV) 3C enzyme is a picornaviral cysteine proteinase involved in the processing of the initially synthesized viral poly protein is therefore important for viral maturation and infectivity. Although it is a cysteine proteinase, this enzyme has a topology similar to those of the chymotrypsin like serine proteinases. Since the enzyme recognizes peptide substrates with a glutamine residue at the P (1) site, a number of ketone-containing glutamine compounds analogous to nano molar inhibitors of cathepsin k were synthesized by Ramtohul \textit{et al}\textsuperscript{265} and tested for inhibition against HAV 3C proteinase. In addition, a 3-azetidinone scaffold was incorporated into the glutamine fragment but gave only modest inhibition. However, introduction of a phthalhydrazido group alpha to ketone moiety gave significant better inhibitors with IC\textsubscript{50} values ranging from 13 to 104 µm, presumably due to the effect of intra molecular hydrogen bonding to the ketone. Lall \textit{et al}\textsuperscript{266} synthesized a number of serine and threonine beta-lactones and were tested against HAV 3C proteinase. The D-N-Cbz-serine beta-lactones displayed competitive reversible inhibition with a K(i) value of 1.50 \times 10^{-6} M. Its enantiomer, L-N-Cbz-serine beta-lactone is an irreversible inactivator with K(inact) = 0.07min\textsuperscript{-1}, K(lota) = 1.84 \times 10^{-4} M and K(inact) / K(lota) = 3800 M\textsuperscript{-1}min\textsuperscript{-1}. Mass spectrometry and HMQC NMR studies using \textsuperscript{13}C-labelled L-N-Cbz-serine beta-lactone showed that inactivation of the enzyme occurs by nucleophilic attack of the cysteine thiol (cys-172) at the beta-position of the oxetanone ring. Although the N-Cbz-serine beta-lactones displayed potent inhibition, other related analogues with an N-Cbz side chain, such as the five-membered ring homoserine gamma-lactones, the four-membered ring beta-lactam, 2-methylene oxetane, cyclobutanone and 3-azetidinone, failed to give significant inhibition of HAV 3C proteinase, thus the importance of the beta-lactone ring for binding has been demonstrated.
2.2.8. Beta-lactam derivatives reduces ethanol consumption in alcohol-preferring rats.

Changes in glutamatergic transmission affect many aspects of neuroplasticity associated with ethanol and drug addiction. For instance, ethanol and drug seeking behavior is promoted by increased glutamate transmission in key regions of the motive circuit. Youssef Sari et al\textsuperscript{267} hypothesized that because glutamate transporter 1 (GLT1) is responsible for the removal of most extracellular glutamate, up-regulation or activation of GLT1 would attenuate ethanol consumption. Behavioral drinking, Statistical analyses revealed a significant reduction in daily ethanol, but not sucrose, consumption following Ceftriaxone (CEF) treatment. During the post treatment period, there was a recovery of ethanol intake across days. Dose-dependent increases in water intake were manifest concurrent with the CEF-induced decreases in ethanol intake. Nevertheless, CEF did not affect body weight. An examination of a subset of the CEF-treated ethanol-drinking rats, on the third day post CEF treatment, revealed increases in GTL1 expression levels within the prefrontal cortex and nucleus accumbens. These results indicate that CEF effectively reduces ethanol intake, possibly through activation of GLT1, and may be a potential therapeutic drug for alcohol addiction treatment.

2.2.9. Beta-lactams decreases acquisition of and motivation to respond for cocaine, but not sweet food, in mice.

No medication is approved to treat cocaine addiction, but mounting evidence by Ward et al\textsuperscript{268} reported that glutamate-directed approaches may reduce cocaine dependence and relapse. The glutamate transporter subtype 1 activator, ceftriaxone, disrupts acquisition of cocaine self-administration, motivation to self-administer cocaine, and conditioned place preference in mice. Repeated ceftriaxone (200 mg/kg) reduced the ability of mice to acquire cocaine and the motivation to self-administer cocaine after successful acquisition without affecting acquisition of or motivation for sweet food. Repeated ceftriaxone had no effect on cocaine-conditioned place preference. These results suggest that a β-lactam antibiotic reduces the direct reinforcing strength of cocaine without producing nonspecific deficits in conditioned learning processes.
2.2.10. Artesunate enhances the antibacterial effect of beta-lactam derivatives.

Yao et al\textsuperscript{269} suggested that although artesunate itself had no antibacterial ability, artesunate significantly increased the antibacterial effect of β-lactam antibiotics against E. coli ATCC 35218. Artesunate increased daunomycin accumulation within E. coli in a dose-dependent manner and reduced the mRNA expression of AcrAB-TolC, an important multidrug efflux system for Gram-negative bacteria. The bacterial number was significantly reduced by as-ODN targeting AcrB, but did not further decrease after additional artesunate treatment. In contrast, artesunate lost its enhancement of β-lactam antibiotics against E. coli AG100A, a strain lacking the gene encoding AcrAB.

2.2.11. Genetic determinants involved in the susceptibility of Pseudomonas aeruginosa to beta-lactam antibiotics.

Alvarez-Ortega et al\textsuperscript{270} suggested that the resistome of P. aeruginosa for three β-lactam antibiotics, namely, ceftazidime, imipenem, and meropenem, was deciphered by screening a comprehensive PA14 mutant library for mutants with increased or reduced susceptibility to these antimicrobials. Confirmation of the phenotypes of all selected mutants was performed by E-test. Of the total of 78 confirmed mutants, 41 demonstrated a reduced susceptibility phenotype and 37 a super susceptibility (i.e., altered intrinsic resistance) phenotype, with 6 mutants demonstrating a mixed phenotype, depending on the antibiotic. Only three mutants demonstrated reduced (PA0908) or increased (glnK and ftsK) susceptibility to all three antibiotics. Overall, the mutant profiles of susceptibility suggested distinct mechanisms of action and resistance for the three antibiotics despite their similar structures. More detailed analysis indicated important roles for novel and known β-lactamase regulatory genes, for genes with likely involvement in barrier function, and for a range of regulators of alginate biosynthesis.

2.2.12. Allergy to beta-lactam antibiotics

Ponvert et al\textsuperscript{271} suggested, studies based on skin and challenge tests have shown that 12–60% of children with suspected beta-lactam hypersensitivity were
allergic to beta-lactams. Responses in skin and challenge tests were studied in 1865 children with suspected beta-lactam allergy to confirm or rule out the suspected diagnosis; (ii) to evaluate diagnostic value of immediate and non-immediate responses in skin and challenge tests; (iii) to determine frequency of beta-lactam allergy in those children, and (iv) to determine potential risk factors for beta-lactam allergy. The work-up was completed in 1431 children, of whom 227 (15.9%) were diagnosed allergic to beta-lactams. Beta-lactam hypersensitivity was diagnosed in 50 of the 162 (30.9%) children reporting immediate reactions and in 177 of the 1087 (16.7%) children reporting non-immediate reactions (p < 0.001). The likelihood of beta-lactam hypersensitivity was also significantly higher in children reporting anaphylaxis, serum sickness-like reactions, and (potentially) severe skin reactions such as acute generalized exanthemative pustulosis, Stevens–Johnson syndrome, and drug reaction with systemic symptoms than in other children (p < 0.001). Skin tests diagnosed 86% of immediate and 31.6% of non-immediate sensitizations. Cross-reactivity and/or cosensitization among beta-lactams was diagnosed in 76% and 14.7% of the children with immediate and non-immediate hypersensitivity, respectively. The number of children diagnosed allergic to beta-lactams decreased with time between the reaction and the work-up, probably because the majority of children with severe and worrying reactions were referred for allergological work-up more promptly than the other children. Sex, age, and atopy were not risk factors for beta-lactam hypersensitivity. In conclusion, it is confirmed in numerous children that (i) only a few children with suspected beta-lactam hypersensitivity are allergic to beta-lactams; (ii) the likelihood of beta-lactam allergy increases with earliness and/or severity of the reactions; (iii) although non-immediate-reading skin tests (intradermal and patch tests) may diagnose non-immediate sensitizations in children with non-immediate reactions to beta-lactams (maculopapular rashes and potentially severe skin reactions especially), the diagnostic value of non-immediate-reading skin tests is far lower than the diagnostic value of immediate-reading skin tests, most non-immediate sensitizations to beta-lactams being diagnosed by means of challenge tests; (iv) cross-reactivity and/or cosensitizations among beta-lactams are much more frequent in children reporting immediate and/or anaphylactic reactions than in the other children; (v) age, sex and personal atopy are not significant risk factors for beta-lactam hypersensitivity; and (vi) the number of children with diagnosed allergy to beta-lactams (of the immediate-type hypersensitivity especially) decreases with time between the reaction and
allergological work-up. Immunologic cross-reactivity of aztreonam with other beta-lactam antibiotics has been studied by Saxon et al\textsuperscript{273} and Rodilla et al\textsuperscript{274}.

2.2.13. Integrated detection of extended-spectrum beta-lactam resistance.

Different ways of bacterial resistance\textsuperscript{278-286} have been shown in (Figure No. 3), Leinberger et al\textsuperscript{275} suggested that extended-spectrum beta-lactamases (ESBL) of the TEM, SHV, or CTX-M type confer resistance to beta-lactam antibiotics in Gram-negative bacteria. The activity of these enzymes against beta-lactam antibiotics and their resistance\textsuperscript{276,277} against inhibitors can be influenced by genetic variation at the single-nucleotide level. He suggested the development and validation of an oligonucleotide microarray for the rapid identification of ESBLs in Gram-negative bacteria by simultaneously genotyping \textit{bla}_{TEM}, \textit{bla}_{SHV}, and \textit{bla}_{CTX-M}. The array consists of 618 probes that cover mutations responsible for 156 amino acid substitutions. As this comprises unprecedented genotyping coverage, the ESBL array has a high potential for epidemiological studies and infection control. With an assay time of 5 h, the ESBL microarray also could be an attractive option for the development of rapid antimicrobial resistance tests in the future. The validity of the DNA microarray was demonstrated with 60 blinded clinical isolates, which were collected during clinical routines. Fifty-eight of them were characterized phenotypically as ESBL producers. The chip was characterized with regard to its resolution, phenotype-genotype correlation, and ability to resolve mixed genotypes. ESBL phenotypes could be correctly ascribed to ESBL variants of \textit{bla}_{CTX-M} (76%), \textit{bla}_{SHV} (22%), or both (2%), whereas no ESBL variant of \textit{bla}_{TEM} was found. The most prevalent ESBLs identified were CTX-M-15 (57%) and SHV-12 (18%).
2.2.14. **Beta-lactam antibiotic inhibits development of morphine physical dependence in rats.**

Rawls *et al*\(^\text{287}\) suggested that β-Lactam antibiotics enhance cellular glutamate uptake. As increased glutamatergic transmission is a primary mediator of opiate dependence, tested the hypothesis that a β-lactam antibiotic (ceftriaxone) prevents development of morphine physical dependence in rats. Morphine (20 mg/kg) was injected twice daily for 10 days to induce physical dependence. Naloxone (10 mg/kg) administration 1, 48, and 96 h after the last morphine injection induced a withdrawal syndrome characterized by the appearance of wet-dog shakes, teeth chattering, eye blinking, jumping, and paw tremor. Ceftriaxone (150, 200 mg/kg) injected once daily during chronic morphine exposure inhibited each naloxone-precipitated withdrawal sign. Ceftriaxone efficacy persisted even after the 96 h-naloxone (10 mg/kg) injection. These results suggest that β-lactam antibiotics inhibit processes leading to development of morphine physical dependence.
2.2.15. **Synergistic antibacterial effect of Beta-lactam derivatives with silver nano-particles.**

Ping *et al*\(^{288}\) The bactericidal action of silver nanoparticles and amoxicillin on *Escherichia coli* is studied, respectively. Increasing concentration of both amoxicillin (0–0.525 mg ml\(^{-1}\)) and silver nanoparticles (0–40 µg ml\(^{-1}\)) showed a higher antibacterial effect. *Escherichia coli* cells have different bactericidal sensitivity to them. When amoxicillin and silver nanoparticles are combined, it results in greater bactericidal efficiency on *Escherichia coli* cells than when they were applied separately.

2.2.16. **Beta-lactam derivatives offer neuroprotection.**

Glutamate is the principle excitatory neurotransmitter in nervous system. Inactivation of synaptic glutamate is handled by glutamate transporter (GLT1). Animal studies show that the GLT1 is important for normal excitatory synaptic transmission, while its dysfunction is implicated in acute and chronic neurological disorders including stroke, brain tumours and epilepsy. It has been discovered that may β-lactam antibiotics are potent stimulators of GLT1, Glutamate transporter are important in preventing glutamate neurotoxicity\(^{289}\).

2.2.17. **Bacteriological antagonism between acylureidopenicillins & cephalosporins, and combination of different antibiotics.**

An antagonism is described by Grimm\(^{290}\) between cefoxitin and azlocillin by means of agar-diffusion test and checker-board titrations of MIC. This phenomenon is attributed to beta-lactamase-induction by cefoxitin. Cefuroxime is less antagonistic, and cefotaxime is indifferent in combination with azlocillin. Combination of mezlocillin and azlocillin with cephalosporin antibiotics has been studied by Neu *et al*\(^{291}\) for their synergistic effects. Amdinocillin in combination with another beta-lactam antibiotic (ampicillin, cephalothin, cefamandole or cefoxitin) was studied by Rosten *et al*\(^{292}\).