Chapter -1 Introduction

Resistance of pathogenic organisms to tolerate antibiotics has become a worldwide problem with severe consequences on the treatment of infectious diseases. The heightened use/misuse of antibiotics in human medicine, agriculture and veterinary is primarily contributing to the phenomenon. There is a perturbing increase of antibiotic resistance in bacteria that cause either community infections or hospital acquired infections. Antibiotic resistance is a problem of deep scientific concern both in hospital and community settings. Rapid detection in clinical laboratories is essential for the judicious recognition of antimicrobial resistant organisms.

Successful application of antibacterial therapy in the critically ill requires an appreciation of the complex interaction between the host, the causative pathogen and the chosen pharmaceutical [1]. The prescriber’s principal desire is to achieve therapeutic drug concentrations at the site of infection, ensuring rapid bacterial killing and the prevention of infection-related organ and tissue damage. Timely recognition of sepsis and the administration of an appropriate antimicrobial agent (with a suitable spectrum of cover) remains the corner stone of effective management [2], but what is more uncertain is the impact of variable antibacterial exposure, beyond simple susceptibility patterns. In this fashion, the host response as well as additional interventions provided can have marked effects on the pharmacokinetics of these agents, leading to highly variable drug concentrations following standard dosing [3–16].

The antibacterial agents have developed resistance to drugs [17]. As we entered the 21st century the prospect for “superbugs” which are resistant to all antimicrobial agents are becoming more of a reality, especially when bacteria are capable of invading the whole of our human body. Therefore, mankind is in great need to develop a new and innovative antibacterial agent to regain the dominance over the pathogenic bacteria. In quest of adequate biological active compounds, a large number of imine has been synthesized. Some of them have found value in therapeutics [18- 19] and also as pesticidal agents [20-21].
Condensation reaction of aldehydes and primary amines are important transformations in organic synthesis as they generate C = N bond to give stable imines, which are used to design medicinal, compounds [22-23]. In mild acidic conditions, imines could be hydrolyzed selectively by tumor cells, as they have lower pH than, cells of normal tissues [24], as a consequence, they have been evaluated for their antiproliferative properties [25-28] against a variety of tumors. Imines and their derivatives have been a research subject [29-37] due to their striking complexometric behaviour and pharmacological characteristics. These properties allow it to play a pivotal role in various biological activities [38-47] viz. antibacterial [48-58], antifungal [59-66] anticancer [67-73] antitubercular [74-76], anticonvulsant [77-79], anti HIV [80-82], antiamoebic [83], anti-inflammatory [84-87], antinociceptive [88], pesticidal [89-90], herbicidal [91] and antimalarial [92]. Imines are also used as pigments and dyes, catalysts, intermediates in organic synthesis, and as polymer stabilizers [93]. Imine linkages are present in various natural compounds and synthetic compounds [94].

Imines (1) having antibacterial and antifungal activities derived from 2-hydroxy-4-methoxybenzaldehyde and ethyl-4-aminobenzoate have been reported by Ilies [95]. Imines (2), has been synthesized via the reaction of salicylaldehyde with N1-(3-aminopropyl) propane-1,3-diamine by Charef [96] et al. exerting a high inhibition of the growth of bacterial strains tested.

\[
\text{(1) \quad \text{ (2)} }
\]

Subsequently, several derivatives constituting azomethine linkage (3) with hydroxy and methoxy substitutions exhibits significant biological activities. Griseofulvin containing three methoxy moieties is a powerful antimycotic agent [97].
Imines (4) derived from 4-substituted and 4, 5 disubstituted -2-aminothiazol and 3-methoxy -4- substituted acetyloxy benzaldehyde were synthesized and screened for their fungicidal activity [98] and found that the presence of methoxy and acetyloxy group in the compound enhances their fungicidal activity.

Recently it has been studied that incorporation of imines of p-amino salicylic acid in the parent compound generates a potent antibacterial agent, as they facilitate the entry of these molecules through lipid enriched bacterial cell wall [99] in *Mycobacterium tuberculosis*.

Literature survey reveals that imines exhibits bacteriostatic and bacteriocidal [100] activity against wide range of Gram positive & Gram negative bacterial strains viz. *C. albicans, E.Coli, S. aureus, B. polymyxa, P. viticola etc.* Naqvi et al. [101] have synthesized imines (5) from 3-chloro-4-floro aniline and several benzaldehydes by non classical methods.
Imine derivatives containing, 4(3H)-quinazolinone moiety can effectively control tobacco and tomato bacterial wilts [102]. Imines (6) & (7), synthesized by sulfathiazole or sulfapyridine with 3-ethoxsalicylaldehyde/ pyridine-2-carbaldehyde/ 2-hydroxy-1-naphthaldehyde, exhibits antibacterial activity against Gram positive bacteria, _S. aureus_ and Gram negative bacteria _E.Coli, Klebsiella sp_ and _P. aeruginosa_ and antifungal activity against _A. niger_ and _Mucor_. Ciprofloxacin and Nystatin were used as standard drug for bacteria and fungus [103].
Domagk’s investigation of compounds containing the sulfonamide functions as potential antibiotics, and has led to a number of very effective antibiotics. The antibacterial compound, Prontosil (8) a dye that strongly stains proteins, showed that it was metabolized to sulfanilamide, a more effective antibiotic. Prontosil is therefore termed a prodrug as it is not the substance, responsible for biological effect but its dose produce the effective drug [104].

\[
\begin{align*}
\text{NH}_2 & \quad \text{N} \quad \text{N} \\
\text{H}_2\text{N} & \quad \text{SO}_2\text{NH}_2
\end{align*}
\]

A group of synthetic organic compounds, derived from sulfanilamide, capable of inhibiting bacterial growth and activity, is called sulfonamides. Sulfonamides are compounds that contains sulfur in a $-\text{SO}_2\text{NH}_2$ moiety directly attached to a benzene ring [105]. Sulfamides, developed in the 1930’s, were the first medications effective against bacterial disease. They appeared as the first "miracle drugs" at a time when death from bacterial infections such as pneumonia and blood poisoning were common [106]. Imine compounds are usually prepared by the condensation of a primary amine (sulfamides) with an active carbonyl compound [107]. The sulfonamide derivatives are widely used in clinical medicine as pharmacological agents with a wide variety of biological actions [108-109]. Sulfonamides are well known for their antibacterial [110-112], antitumour [113], diuretic [114], and antithyroid [115] activities. The presence of azomethine and sulfonamide functional group is responsible for biological activity.

In a study, imines (9) & (10) were synthesized by condensation of 4-aminobenzenesulfonamide and carbonyl molecules, their biocidal activities were also evaluated against \textit{S. aureus}, \textit{B. subtilis} and \textit{E. coli}, \textit{P. aeruginosa} and for their fungicidal activities against \textit{C. albicans} [116-117].
Synthesis and *in-vitro* antimicrobial activities of new imines obtained from 3-formyl chromone with sulfapyrdazine, 3-formyl-6-methyl chromone with sulfamethoxypyridazine and 3-formyl-6-methyl chromone with sulfaproxylene were have been investigated by Hadi [118] et al. A series of 1,5-dimethyl-2-phenyl-1,2-dihydro-3H-pyrazol-3-one containing imines have been synthesized and were screened for their antibacterial activities[119]. A series of N-(1, 3-benzothiazol-2-yl)-2-[(2Z)-2-(substituted arylidene) hydrazinyl] acetamide (11) have been synthesized by Soni [120] et al. and determined their antimicrobial activities *in-vitro* against Gram-positive and Gram-negative bacteria. Various heterocyclic imines, having O, N and S donor atoms, have been reported by several scientists [121-123]. Imines (12) formed by the condensation of 2-thiophenecarboxaldehyde with 2-aminopyridine, N-(2-thienylmethy-thyl-idene)-2-aminopyridine have been reported by Spinu [124] et al.

Imines have a wide range of biological activity against pathogens [125]. Thanassi et al. reported imines activity against tuberculosis [126]. Heterocyclic imines derivatives have been also synthesized and their anticancer, antiamoebic, antibacterial, antifungal, antiviral and anti-HIV activity have been reported [127-128]. It has been reported by various researchers that imines have potential applications in
anti-inflammatory, anti-proliferative and antipyretic properties [129–131]. Azomethins are used as intermediates for the formation of different type of heterocyclic compounds by a nucleophilic addition reaction. Imine derivatives containing heterocyclic rings, e.g. pyrazole, isoxazole, thiazole, and β lactam exhibits considerable biological activities, such as, antimicrobial [132], antiamoebic [133], antinociceptive [134], anticancer [135], antidepressant and anti-inflammatory [136].

Further discoveries, designing and synthesis of imines through viable approaches have explored antimicrobial activity [137-144]. Imines have been reported as potent antitumor agent [145-147]. Imine derived from vanilin as the central molecule with morpholine(13) and sulfamethoxazole(14) as the side chain, showed marked inhibition against the investigated bacteria and appeared to be promising antimicrobial agents [148-149].

The important physical and biological properties of the imines are directly related to intramolecular hydrogen bonding and proton transfer equilibria. Unfortunately most imines are chemically unstable and show a tendency to get involved in various equilibria like tautomeric interconversions, hydrolysis or formation of ionized species, therefore successful application of imines requires a careful study of their characteristics. The imines (15) prepared by condensation of aromatic aldehyde derivatives with aromatic mono and diamine derivatives have been used for monitoring pH and as optical sensors [150].
Active and well designed imines ligands are also considered 'privileged ligands' due to their wide application in the field of synthesis, catalysis, material chemistry, and as models for biological systems. Imines of $p$-substituted aniline (16) and its complex have a variety of applications in biological, clinical and analytical fields [151]. The imines works as a ligand then the complexes are found to be antimicrobial active [152].

Other imines derivatives, which possess antibacterial activity, are: benzimidazole, thiazole, pyridine, glucosamine, pyrazolone, hydrazide, thiazolidiones, indole, thiosemicarbazone, & $p$-fluorobenzaldehyde [153]. Isatin imines ligands are marked by antiviral activity, and this fact is very useful in the treatment of HIV [154]. Cryptolepine, valid indolchinoline alkaloid, isolated from African plant Cryptolepis sanguinolenta, also used in the treatment of malaria, is the product of multi-stage reaction, in which imines has been involved [155]. Azomethine derivatives of N-hydroxy-N’-aminoguanidine block ribonucleotide reductase in tumour cells, so that they are used in the treatment of leukemia [156].

Imines are readily hydrolyzed under acidic conditions leading to active aldehydes which can act as alkylating agents [157] and diuretic activities [158]. Some heterocyclic and aliphatic amines have exhibited biological significance such as
antimetabolites of pyridoxal phosphate [159], bacteriostatic activity [160], and chorismate synthase inhibition [161]. Imines are utilized as starting materials in the synthesis of industrial [162-163] and biological compounds [164-165]. The variety of biological activities of substituted-1, 3, 4-oxadiazoles include anti-inflammatory [166], hypoglycaemic [167], antianxiety, antidepressant [168] and antimitotic activities [169].

Researchers have reported imines of 2, 5-disubstituted -1, 3, 4-oxadiazoles (17) for their antimicrobial activities [170]. A New series of imines derived from benzimidazole derivatives showed potential antimicrobial activities [171]. The presence of an alkyl chain at the p-position of the aldehyde and aniline fragments of imines has been of importance and favours the existence of liquid crystal phases [172-173]. N-benzylidene aniline imines are important in exhibiting thermochromism and photochromism [174]. In view of the importance of these compounds, chemists generated the imines derivatives (18) by introducing different substituents into the existing skeleton of the molecule.

![Image of chemical structures](image-url)
substitution and its position [176] on the phenyl rings of aromatic aldimines on the biological activity. The synthesis and assaying of biological activity of imines have received considerable interest [177]. Presence of C=N bond provide a potential site for chemical [178-179] and biological activities [180-181] in aldimines and ketimines.

Enhanced activity of vanillin azomethines more than intermediates in 2,3-bis(3-methoxy-4-butoxybenzaldehyde)diamino pyridine (Azomethine 19) and 2,3-bis(3-methoxy-4-carboxymethylbenzaldehyde) diamino pyridine (Azomethine 20) has been found due to -CH=N- group prepared by Konstantinovic [182] et al. and tested against Candida albicans, Candida lipolytica and Sacharomyces cerevisiae.

\[
\text{R}^1 = -\text{C}_6\text{H}_9 \text{ (Azomethine 19) OR} \\
\text{R}^2 = -\text{CH}_2\text{COOH (Azomethine 20)}
\]

\(\beta\)-lactam is a lactam with a heteroatomic ring structure, consisting of three carbon atoms and one nitrogen atom. A lactam is a cyclic amide. \(\beta\)-lactam antibiotics have been the most widely used antibiotic drugs for more than 80 years and still constitute the most important group of antibiotics. The heterocyclic compounds are of considerable interest because of their chemistry and biological activity. \(\beta\) -lactam (21) is one of the most important heterocyclic compounds in medicinal chemistry, since it forms a part of antibiotic molecule [183].
Azetidin-2-ones represent a unique ring system, with interesting chemistry and great biological potential. Besides its well known antibiotic activity, β-lactam ring system exhibits a wide range of activities. The biological and pharmacological profile of azetidin-2-ones is reviewed [184]. β-lactam derivatives were synthesized by cyclization of imines. After cyclization, the less active imines became active and more antibacterial [185].

The new cis-β-lactams synthesized by [2 + 2] cycloaddition reactions of chiral imines with a chiral ketene have been investigated. The cycloaddition reaction was found to be totally diastereoselective leading to the formation of the cis-β-lactam derivatives. The newly synthesized cycloadducts (22) exhibits antimalarial activities against *P. falciparum* K14 resistant strain [186].

![Chemical structure of compound 22](image)

Desai et al. have synthesized azetidinone derivative 7-(4-(4-(2-benzoylehydrazinyl)-6-(2-chloro-3-oxo-4-substituted benzaldehydeazetidin-1-ylamino)-1, 3, 5-triazin-2-yl) piperazin-1-yl)-1-cyclopropyl-6-fluoro-4-oxo-1,4-dihydroquinoline-3-carboxylic acid (23) and evaluated biologically for antimicrobial activity [187]. Synthesis of N-(4-(7-(3-chloro-2-oxo-4-(4-(Methoxy/trifluoromethyl) phenyl) azetidin-1-yl)-4-((2R, 6R)-2,6-dimethylmorpholino)quin azolin-2-yl)phenyl) piperidine/Morpholine/Thiomorpholine/N-Methylpiperazine)-1-carboxamide derivatives (24) of 2-Azatidinone reported recently by Prabhakar[188] et al.
The reaction of amines with aldehydes and ketones has numerous applications such as identification, detection and determination of aldehydes of ketones. The reaction also finds utility in purification of carbonyl or amino compounds, e.g. amino acids in protein hydrolysates [189] or for the protection of these groups during complex or sensitive reaction [190]. It has been suggested that azomethine linkage (CH = N-) is responsible for augmenting the biological activity of imines [191-194]. Imines from 5-chloro salicylaldehyde, 5-bromo salicylaldehyde, 4-N, N-dimethylamino benzaldehyde have been reported to show encouraging tuberculostatic activity [195]. Imines bases have pronounced biological activities [196-197].

A series of new imines and amines have been synthesized by condensation of 1H-3-ferrocenyl-1-phenylpyrazole-4-carboxaldehyde with the corresponding amines, followed by reduction with sodium borohydride. The synthesized compounds have been screened for their in vitro antimicrobial activity [198]. The important progression of the bacterial infections and the recrudescence of resistance to antibiotics used currently encourage the researchers to look for new active molecules [199].

Until 1970 most of the β-lactam antibiotic chemistry was revolving around either penicillin or cephalosporin. This isolation of 7-β-methoxycephalosporins [200] from streptomyces in 1971 stimulated the search for
novel β-lactam antibiotics from microbes. This extensive quest for novel β-lactam skeleton has led to the isolation of active antibiotics not only from eukaryotic fungi, actinomycetes, but also from bacteria and fungi. This has led to the expansion of β-lactam antibiotic family to an ever increasing number. In the past century, the design and development of β-lactam antibiotics has been highly influential within drug discovery, due to their biological and pharmacological activity. β-lactams are the most well known and most widely used of all naturally occurring antibiotics and are secreted by moulds of the genus *Penicillium*. World’s first commercially available antibiotic was originally synthesised by Josef Klarer and Fritz Mietzsch, colleagues of Domagk at the Bayer Company in Germany [201].

An equimolar reaction of the *N*-salicylidenebenzhydrylamine with diphenylketene afforded a mixture of 2-{[(benzhydrylimino) methyl] phenyl-2,2-diphenylacetate and 1-benzhydryl-3,3-diphenyl-4-[2’-(o-diphenylacyl)hydroxyphenyl]-2-azetidinone. The reactions of various *N*-salicylideneamines with 2.2 molar equivalents of 2-diazo-1,2-diarylethanones have been carried out to afford 1-substituted-3,3-diaryl-4-[2’-(O-diarylacyl)hydroxyphenyl]-2-azetidinones (25) in excellent yields. In a study by Jubie [202] et al, *p*-anisidine was condensed with different substituted aromatic aldehydes to form respective imines. The imines were cyclised with chloroacetylchloride in triethylamine to yield the corresponding 2-azetidinones (26).
Sulfonamide [203-207] drugs were the first effective chemotherapeutic agents to be employed systemically for the prevention and cure of bacterial infection in human beings. Based on these observations some fluoro substituted sulphonamide benzothiazoles have been synthesized and incorporated with azetidinones [208,209] in the hope of getting pharmacological agents with broad spectrum of anti-mycobacterial activity [210]. N-sulfonyl monocyclic β-lactams is a group of monocyclic β-lactams [211-212]. N-sulfonyl β-lactams have been shown to be highly useful compounds for medicinal chemistry [213]. Numerous articles can be found throughout the literature describing the preparation and use of N-sulfonyl β-lactams as intermediates in the synthesis of other target molecules [214]. A large numbers of N-sulfonyl-β-lactams have been examined for biological properties [215]. Konaklieva et al. have synthesized the N-sulfonyl-β-lactams tested them against some bacteria [216]. It appears that the nature of the substituents has many crucial roles to play in bioactivity.

A series of novel azetidinone derivatives showing moderate to good antimicrobial activity against some bacteria and fungi have been synthesized from the intermediate imines by James et al. [217]. Cyclocondensation of imines with acetylichloride resulted in the formation of azetidinone derivatives (27). Recently, 5-[o-(N-benzyl) phenyl sulfamido]-2-[3-chloro-4-(p-dimethylamino) phenyl-2-oxo-azetine-1-yl] amino-1,3,4 -oxadizole (28) and their derivatives have been reported by Naser [218] et al.

![Image](27)
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Synthesis of 1-(substituted benzylidene)(N-sulphonyl hydrazino)-2-(N-methyl-phthalyl)-benzimidazole imines and 1-(N-sulphamido)-[2'-(N-methyl-phthalyl)-benzimidazoyl]-(3-chloro-4-(substituted phenyl)-azetidin-2-ones (29) have been reported by Pramilla [219] et al. 2-[2(2,3-Dimethoxyphenyl)-1-methanesulfonyl-4-oxo-azetidin-3-yl]-4-nitroisoindole-1,3-dione (30) monocyclic β-lactam derivatives, active against *S. aureus*, *B. subtilis*, *E. coli*, and *P. aeruginosa* were synthesized by Jarrahpour [220] et al.

Azetidin-2-one derivatives containing aryl sulfonate moiety reported with anti-inflammatory and anti-microbial activity. The synthesis, involved reaction of 2-hydroxy benzaldehyde with *p*-toluidine sulfonyle chloride and further on reaction with *p*-aminobenzoic acid or 2-aminopyridine, corresponding aldimines were formed. The aldimines on reaction with chloroacetyl chloride resulted in the corresponding
azetidin-2-ones [221] (31). Sulfa drug moiety containing new azetidinone derivatives owing good antimicrobial activity: 4-[3-chloro-2-(5-nitro-furan-2-yl)-4-oxo-azetidin-1-yl]-N-pyrimidin-2-yl-benzenesulfonamide(32) have been prepared by Salman [222] et al. via cyclocondensation of the imines derived from sulfa drugs with chloroacetyl chloride in the presence of triethylamine. The imines were prepared by the condensation reaction of the sulfa drugs, (sulfadiazine and sulfanilamide) with the 5-nitro-2-furancarboxaldehyde.

![Chemical Structures](image)

- **Synthesis of precursors**

  In view of the immense significance of the pharmacological and biological activities associated with aromatic aldehydes we described here the synthesis and characterization of 3-methoxy 4-acetyloxy benzaldehyde and 3-methoxy-4-p-toluene sulphonyloxy 5-allyl benzaldehyde. These aldehydes have been synthesized as the precursors for the preparation of imines.

  - 3-methoxy 4-acetyloxy benzaldehyde (33) has been synthesized by acetylation of vanillin in presence of sodium hydroxide.
3-methoxy 4-<em>p</em>-toluene sulphonyloxy 5-allyl benzaldehyde (34) has been synthesized by allylation, Claisen rearrangement and tosylation of vanillin.

- **Synthesis of imines**

  Synthesised aldehydes (33&34) have been condensed with several sulpha drugs for the synthesis of two series of imines (a1-a6) & (b1-b6).
R=Sulpha drug variants

- **Synthesis of 2-azetidinone analogs**

4-membered, β-lactam ring has been incorporated on the imine linkage of newly synthesized imines (a1-a6) & (b1-b6) in order to synthesise different imine analogs (c1-c6) & (d1-d6).
Antimicrobial Screening of imines & β-lactams

The synthesized compounds were screened in-vitro against, selected three bacterial strains namely E. coli, Pseudomonas aeruginosa, Bacillus subtilis and two fungal strains namely Aspergillus niger, Aspergillus flavus by using disc diffusion method.
References:


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