Chapter 18

Antibacterial lead compounds and their targets for drug development

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Chapter outline

18.1 Introduction

Since antiquity, natural bioactive compounds have been a major source of drugs and protect against countless health challenges affecting mankind. Herbs, microbes, and marine organisms produce wide variety of bioactive compounds, which are beneficial in drug preparation, lead structure, or raw materials as well [1]. The previously documented records on Greek, Chinese, Roman, Egyptian, and Indian traditional medicine systems have described the plentiful aspects of therapeutic plants and advice for use in the management of various abnormalities. Even in developing countries, plant-based medicinal formulation are been passed verbally from generation to generation. It has been observed that nearly 14%–28% species of higher plants are used as medicine, while the remaining 72% are found to be suitable after following up on ethnomedicinal use of their bioactive compounds. There are plentiful examples of natural antiinfective drugs [2]. Isoquinoline, heterocyclic aromatic alkaloidal drug isolated from *Cephaelis ipecacuanha* and other associated species, has been used as amoebicidal agent along with hepatic abscesses treatment caused by *Entamoeba histolytica* infections. Quinine occurs naturally in the bark of *cinchona* tree. Besides its sustained effectiveness in the management of malarial

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infection, it can also be used in nocturnal leg cramps medication. Presently, the commonly recommended drugs are quinine analogs such as chloroquine.

It is validated that plant and their derivatives are present in or act as a suitable model for 50% Western drugs [3]. Various established drugs used in the current medicinal system were primarily used in crude form in traditional therapeutic practices or for various other purposes that supported effectively beneficial biological activity. The usual benefit of using plant-derived drugs is that they are comparatively safer than synthetic substitutes, providing thoughtful medicinal advantages and more reasonable treatment. There are usually two major routes of drug discovery: the first one refers to the formation of completely new chemicals and analyzing them for a particular pharmacological interest. The other method is to recognize the biological or chemical source and examine it for direct or indirect involvement in the formation of new drug. The 19th century was stated as the golden era for the development of synthetic drugs. A number of people became attentive toward synthetic drugs because of their rapid action and cost-effective bulk production by industries. In the 1970s, around 75% of all standard medicines are either derived synthetically or as the product of fermentation. Lastly, the enhanced number of occurrences of microbial resistance toward synthetic formulations and microbe-derived antibiotics has turned the attention of scientists toward the traditional medicines, especially herbal drugs or drugs of plant origin [4].

18.1.1 Characterization of good antibiotic

The antibiotic class that has been most successful in terms of medical utility is the β-lactam class followed by fluoroquinolones and the macrolides, with rifampicins, aminoglycosides, and tetracyclines, secure minor but an imperious positions. Nearly all are derived naturally (even though their marketed forms are usually semisynthetic or fully synthetic), but the fluoroquinolones are synthetically prepared compound. Fluoroquinolones and β-lactams (bactericidal) are pathologically the most potent drugs and are commercially available. Meanwhile, rest are the temporarily bactericidal (aminoglycosides and rifampicins) or bacteriostatic (macrolides and tetracyclines). Their key targets are enzymes, which are connected with essential cellular utilities, viz. dynamics of nucleic acid supercoiling in replication and transcription (fluoroquinolones), peptidoglycan synthesis (β-lactams), DNA to RNA transcription (rifampicin), and mRNA translation for protein formation (macrolides, aminoglycosides, and tetracyclines). Besides this, each antibiotic class prevents one major cellular activity; some drugs possess multiple molecular targets such as fluoroquinolones interact with DNA topoisomerase IV and DNA gyrase (two separate type II topoisomerases). From the above details, two major features arise. Firstly, the antibiotics discontinue major metabolic pathways in bacteria. Secondly, the antibiotics are those molecules which can be modified accordingly for more effective antibacterial properties and to gain good pharmacodynamic/pharmacokinetic attributes with least acceptable toxicity against a living being cells.

18.1.2 Criteria for choosing and validating a suitable antibiotic target

An enzyme should be a best suitable target for drug, as they played a major role in various biochemical processes or pathways within bacterial cells. Several complementary methodologies have been used to identify genes coding for important target functions in related pathogenic species. Genetics is the initial step to understand the necessity of particular genes, commonly by inadvertent transposon mutagenesis or by constructing systematic knockouts for gene by gene. Information of complementing gene essentiality is a useful tool for analysis of pathogen genome sequence to collect a list of genes that are conserved between major pathogens. These targeted genes should be absent or expressively modified in the human genome. The purpose is to identify novel and vital targets existing in most curious pathogens, whose prevention will not cause any collateral harm to the human host. Additionally, there are numerous limitations and critical points to investigate before restricting the search for ideal drug hits.

For instance, aminoglycosides, macrolides, and tetracyclines directly interact with the ribosome to avert synthesis of protein. Although the resistance to these antibiotics, at the level of structural modifications in the ribosome, does not suggest resistance against either of the others, it may be because of the different molecular targets. Indeed, various other antibiotics also damage the ribosomal machinery at structurally different targets. The purpose is that when preparing a data of active target sites, one should not remove those targets for which effective antibiotics already developed because there may be some separate methods of preventing the functional entity. Hence, the major features which researchers want to identify in a novel antibiotic target interaction are that it must prevent the bacterial growth, should be accessible in a bulk amount of key bacterial pathogens, and should not be altered by previously using resistance mechanisms. The preconception to evade mammalian cell targets also comes with an important attention. Ribosome occurs in all cell types and its large subunit is extremely conserved at both the organizational and operational levels. In authenticity, the ribosomal

molecular machine is one of the key target sites that we know for natural antibiotics. Another effective target site selection is the chance that resistance due to a target modification should come with a noteworthy fitness cost, but this is presently challenging to forecast in advance.

18.1.3 Targets of major antibacterial drugs

To reveal the mechanism of antibiotics action and why they become less effective, there is a need to discuss the main classes of target sites. There are commonly three identified targets for the major antibacterial drugs, namely bacterial protein synthesis, cell wall biosynthesis, and DNA replication and repair.

18.1.4 Protein synthesis

The prokaryotic ribosomal RNA and associated protein machinery are much enough distinct from the analogous eukaryotic machinery as there are various protein synthesis inhibitors, targeting separate steps in ribosome action along with desire antibacterial activity. These encompass essential antibiotics like macrolides of the class erythromycin [5], the aminoglycosides [6] (streptomycin was an initial member of this class which was later displaced by synthetic alternative kanamycin), and tetracyclines [7] (an aromatic polyketide biosynthetic pathways products) (Fig. 18.1). There are a number of steps involved in protein assembly, viz. initiation, elongation, and termination by the ribosomal machinery that could be prohibited by these drug and various other protein synthesis inhibitors. This diversity suggests that protein synthesis will provide a multidimensional target for new antibiotics [8].

18.1.4.1 DNA replication and repair

The fluoroquinolones (e.g., ciprofloxacin) (Fig. 18.2) are synthetic antibiotic that prevents bacterial proliferation by preventing DNA gyrase activity [9]. This enzyme requires for an unwinding of DNA double-strand coiling that introduced after each round of DNA replication. DNA topoisomerases are categorized into type I or II classes on the bases of their mode of action. Bacterial DNA gyrases come under the type II topoisomerases and the transient break of both DNA strands comprises the alterable attachment on the sliced DNA at the 5′ ends to tyrosyl residues on each of the two GyrA subunits [10]. Ciprofloxacin, a quinolone antibiotic, is a potent suppressor of DNA gyrase and proceed by creating a complex structure with the enzyme and cleaved DNA that is covalently joined to the GyrA subunits. Type-II topoisomerase also acts as an active target and perhaps the major one in *Staphylococcus aureus* infections [11]. In each of the three main targets, namely cell wall synthesis, DNA replication, and protein biosynthesis, the antibiotics use differs biochemically between prokaryotic and eukaryotic machinery to act specifically. New classes of antibiotic drug that may work on surplus and new targets will have to show equivalent medicinal indices and efficiency to toxicity ratio to achieve regulatory and extensive approval.

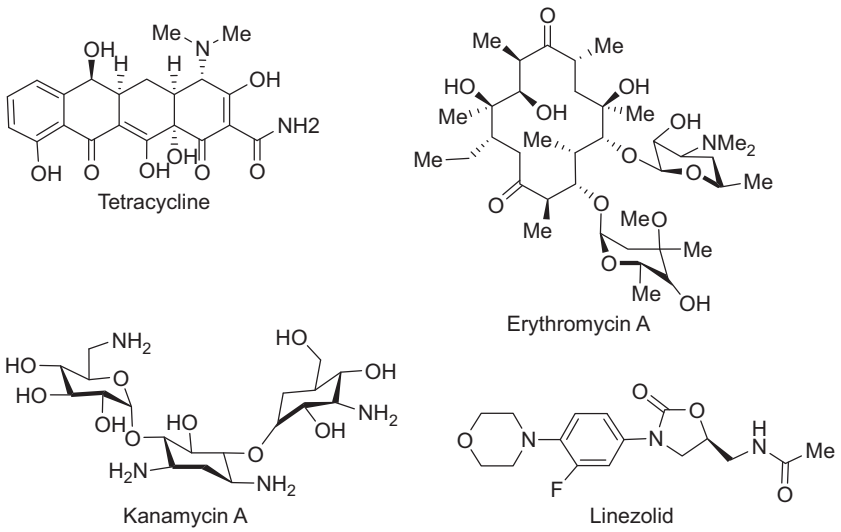
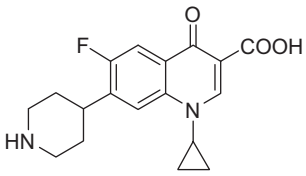


FIGURE 18.1 Antibiotics comprises a vast range of natural product structures, demonstrated by tetracyclines, erythromycin (class of macrolides), kanamycin (aminoglycosides), and linezolid (oxazolidinone). These drugs act on the 23S rRNA and related proteins in the peptidyl transferase center of the ribosome to block steps in the elongation of the protein chain.



Ciprofloxacin

FIGURE 18.2 The fluoroquinolones, denoted by ciprofloxacin, destroy microbes by preventing DNA gyrase and the associated topoisomerase IV in the midcatalytic cycle, by trapping a double-cleaved DNA intermediate.

18.1.4.2 Aminoacyl t-RNA synthetases

Just before the joining of polypeptides, amino acids make the association with particular t-RNA molecules. Nearly in all organisms, each amino acid is transformed by a particular aminoacyl tRNA synthetase (E) to an aminoacyl adenylate-enzyme complex (E-AA-AMP), which then make binding with an amino acid—specific t-RNA to generate an aminoacyl t-RNA (AA-tRNA) molecule. AA-tRNA molecules are then arranged in a linear manner by forming a complex with ribosome-bounded mRNA. Authentication of the aminoacyl tRNA synthetases as effective drug targets has been supported by the discovery and advancement of mupirocin (pseudomonic acid), a suppressor of bacterial isoleucyl tRNA synthetase (IRS) [12]. Several other AA-tRNA synthetases are crucial for microbial growth and feasibility and other inhibitors of synthetase with antimicrobial action have been reported. Furthermore, most of them are deficient in prokaryotic specificity or having the least antibacterial property. This possibly reflects insufficient uptake into bacteria, which has resulted in chemical derivatization programmes to seek analogs with improved antibacterial potency. Indolmycin was identified in 1960, which is now gaining attention as an effective antibacterial candidate [12,13]. As the crystal forms of different AA-tRNA synthetases have now been understood, there are new prospects for structure-based design of inhibitors against this enzymes family.

18.1.4.3 Biosynthesis of cell wall

The strength of bacterial cell wall is connected with the peptidoglycan, a web-like network of glycan and peptide strands that can be interconnected covalently. The larger fragment of neighboring peptide strands is linked with amide bond and the reaction is mediated by transpeptidases. Transglycosylases lengthen the sugar chains by adding new peptidoglycan units from *N*-acetylglucosamine-b-1,4-*N*-acetylmuramyl-pentapeptide-pyrophosphoryl-undecaprenol (lipid II). Bifunctional enzymes cover both the transpeptidase and transglycosylase domains that are the target sites for bacterial assassination by β-lactam bearing cephalosporins and penicillins, which acylate transpeptidases active sites (also termed as penicillin-binding proteins or PBPs) [14] and act as pseudo-substrates. The opened ring, penicilloylated transpeptidases, gradually deacylated and thus occupied the active sites of enzyme, averting general peptide chains cross-linking with the peptidoglycan layer and exit it instinctively weak and vulnerable to damage on alteration in osmotic pressure. Besides cephalosporin and penicillin, the vancomycin family of glycopeptide antibiotics also targets the peptidoglycan layer. Instead of targeting the enzymes functioning as cross-linking of peptide, vancomycin bind with peptide substrate [15] and by doing so inhibit it from reacting with either transglycosylases or transpeptidases. The final result is the same as incapable in the formation of peptidoglycan cross-links, which leads to a fragile cell wall that predisposes the treated bacteria to lysis. The cup-shaped structure of vancomycin antibiotic form five hydrogen bonds with D-Ala-D-Ala dipeptide terminus of each uncross-linked peptidoglycan pentapeptide side chain, which is responsible for the high affinity of antibiotic for its target, both in lipid-II intermediate and in partially cross-linked walls. As vancomycin and β-lactams work on contiguous manner as substrate and enzyme, they show interaction when used in combination.

18.1.5 Potential targets (new possible antibacterial targets)

18.1.5.1 Two-component signal transduction systems

Two-component signal transduction (TCST) system is ubiquitous in prokaryotes and naturally contains a histidine kinase linked with membrane and a response regulator present in the cytoplasm. Microbes use this system to observe the changes in the surrounding environment and acclimatize accordingly, and thus they can survive. Various TCST systems had been recognized till the beginning of genomic era in which some had been worked in pathogenesis, and their efficacy as novel antibacterial targets was recommended. However, little information was known related to the number of different systems

that occurred in a particular pathogen. Genomic sequencing disclosed the complete set of TCST systems in diverse microbial species such as *Helicobacter pylori*, *Haemophilus influenzae*, *Streptococcus pneumoniae*, and *S. aureus*. Detailed computational evaluation of these systems suggested that homology occurs between the TCST systems within the same microbes and from microbes to microbes and that no human homologous exists. Additionally, the requirement of the complete set of TCST systems in *S. aureus* and *S. pneumoniae* has been shown that several systems are needed for the handling of infections [16,17]. Therefore, the search of a wide spectrum and the specific inhibitor of TCST systems would compromise the microbe's capability to acclimatize and survive in any type of surrounding atmosphere, thus forming a striking antibacterial strategy. Till now, no targeted inhibitors of TCST systems have been prepared, but the future accomplishment of this area is based on the design of physiologically relevant, high output relative to input assays for the major systems. Genomics has emphasized TCST systems as a valid approach for novel antibiotic discovery.

18.1.5.2 FtsA–FtsZ interaction

Cell division is crucial and a critical process for the survival of microbes, yet it is perhaps one of the least fully disclosed process. Cell division includes a protein to protein communication network that is essential for the development of septum. FtsZ played a key role in this network as it forms a ring-shaped structure at the mid of the cell by the process of polymerization, after that it added other proteins to the polymer to trigger the downstream actions in the cell division. A number of evidence indicated that protein to protein interactions are crucial for cell feasibility and hence suitable candidate as antibacterial targets, and FtsA–FtsZ protein interaction is one of them. In the last few years, extensive efforts in this area had focused on the proteins associated with cell division in *Escherichia coli*. Study of the FtsA and FtsZ proteins from diverse bacterial genomes has suggested that these are ubiquitously distributed and conserved proteins, which are still studied to evaluate these proteins in Gram-positive pathogens. Arrangement of FtsZ sequences in different pathogenic species indicates a conserved motif toward C-terminus end of protein, which might actively participate in interacting with other proteins used in cell division. Site-directed mutagenesis of this section in *S. aureus,* FtsZ recognized as a single important residue for interaction with FtsA in *S. aureus* [18]. This study revealed that the interaction between FtsA and FtsZ might be relatively small and hence, the formation of small molecule antagonists may be worthwhile during antibacterial approach. There are several other vital interactions necessary for cell division that provides importance to the investigation, and the task will be to design an efficient high-output screening procedure for such types of interactions.

18.1.5.3 Chorismate biosynthesis

Chorismate biosynthetic pathway is necessary for the formation of p-aminobenzoic acid ubiquinone, aromatic amino acids, enterochelin, and vitamin K. Mammals do not hold such type of pathway, and therefore, it is a selectively preferable antibacterial target. It is well-known that bacteria cannot utilize the end product of this pathway from the host and that product is crucial for bacteria to grow in vivo and sustain an infection. The necessity of the pathway has been described predominantly in Gram-negative microbes, through which deletion or mutation in one of the genes responsible for biosynthesis, such as 5-enolpyruvylshikimate-3-phosphate (EPSP) synthase, has been revealed to give rise to extremely virulence-attenuated strains in vivo. Furthermore, earlier to the misuse of bacterial genomics, the vast mainstream of work on the biosynthetic enzymes and investigational antimicrobial targeted at this pathway focused on *E. coli*. Still, investigation of *S. pneumoniae* and *S. aureus* genomes has demonstrated the occurrence and maintenance of the genes involves in chorismate biosynthetic in major Gram-positive pathogens. This has assisted in the identification and specificity testing of such type enzymes in Gram-positive pathogens. For instance, the deletion of the aroA gene, which codes for EPSP synthase causes the strain to be entirely weakened for pathogenicity in two separate animal models with no retrievable bacteria. *S. pneumoniae* EPSP synthase has been broadly categorized [19] and a vast output screening layout has been designed. If the data obtained from this enzyme are illustrative of the other enzymes in the Gram-positive pathway, then biosynthesis of chorismate delivers an additional source of wide spectrum antibacterial targets.

18.1.5.4 Isoprenoid biosynthesis

There are fundamentally two types of pathways through which biosynthesis of isopentenyl diphosphate (IPP) have been defined: the traditional 'mevalonate' pathway and the more recently recognized 'nonmevalonate' pathway, which does not synthesize IPP with the help of mevalonate. The relative genomic investigation has recognized the whole set of mevalonate pathway enzymes in *Enterococci, Streptococci*, and *Staphylococci*. The Gram-positive cocci forms of these genes are extremely different from the mammalian equivalent, delivering rationale for the prospective selectivity of this target area.

Additionally, genetic interruption investigations have shown that five of the six genes codes for the pathway are important for the in vitro development of *S. pneumoniae*. Subsequently, this study has established a complete vital selective pathway appropriate for antibacterial targets [20,21].

18.1.5.5 Fatty acid biosynthesis

In higher eukaryotic organisms, the biosynthesis of fatty acid is governed by a single polypeptide chain, while in case of microorganisms, the pathway is mediated by various distinct enzymes. This difference in fatty acid biosynthesis makes microbial pathway a selectively potential antimicrobial target. Before genomic analysis, much of the work related to this pathway and the constituent enzymes was studied on *E. coli* [22]. Furthermore, extensive work in *E. coli* had revealed that triclosan, diazaborine, thiolactomycin, and cerulenin destroy microbes via inactivation of few or more of the enzymes in this pathway. This provides some earlier indication of the potential targets of this area for recognizing antibacterial agents. Prior information to completely sequenced and proper assembled microbial genomes has permitted the characterization of the constituent enzymes in a diversity of essential clinical pathogens, which delivers a clear investigation of the effective spectrum of a specific enzyme [23]. For instance, FabK would be a suitable target for *streptococcus*, while the existence of FabH in all the key RTI and Gram-positive pathogens makes this target suitable for broad-spectrum usefulness. The efficacy of the enzymes used in the biosynthesis of fatty acid as novel antibacterial targets has lately been validated with FabI. High-throughput selection of this enzyme has provided two unique antibacterial targets. Both of these targets prevented *E. coli* and *S. aureus* FabI enzymes. Advanced analysis of the imidazole leads to 16-fold enhancement in antibacterial action and a five-time improvement in efficacy against the enzymes [24]. Furthermore, strains with adjustable expression levels of FabI have been used to track and validate that the mechanism of antibacterial action of these compounds is required via prevention of FabI. These leads suggested the prospectivity of screening Fab enzymes to discover new antibiotics for the future.

18.1.6 Major groups of natural antimicrobial compounds

18.1.6.1 Phenolic compounds

Phenolic compounds are the simplest known bioactive phytochemicals that consist of a benzene ring with single hydroxyl group. Caffeic and cinnamic acids are key representatives of an inclusive collection of phenylpropane-derived compounds that are in the maximum oxidation state, well-established to possess antimicrobial activities [25]. Both pyrogallol and catechol are hydroxylated phenolic compounds supposed to be toxic against microbial agents. Augmented hydroxylation of the phenolic group has been suggested to outcome in amplified toxicity toward microorganisms. The numbers and the site(s) of hydroxyl (OH) groups on the phenolic group are assumed to be associated through their relative toxicity toward microorganisms, with proof that high hydroxylation results in enhanced toxicity [26]. Consequently, in some cases, it has been observed that highly oxidized phenolic compounds can act as an inhibitor [27]. Phenolics are thought to prevent microbial enzymes maybe by reacting with sulfhydryl groups (the oxidized phenols) or by nonspecific associations with the proteins [28]. Phenolic compounds having a side chain of three carbons at a lower oxidation level and comprising no oxygen are categorized as essential oils and frequently mentioned as antimicrobial as well. Eugenol is a well-classified compound isolated from clove oil (Fig. 18.3A). Eugenol is supposed to be a good bacteriostatic against both bacteria and fungi [29,30].

18.1.6.2 Quinones

Cell wall polypeptides, adhesin molecules present in the cell surface, and membrane-bound enzymes are possible targets for the quinones (Fig. 18.3C). It forms an irreversible complex with nucleophilic amino acids present in proteins [31], therefore leading to protein denaturation and loss of its activity. Anthraquinones, one of the leading class of quinones, have been revealed to possess antibacterial potential in *Bacillus subtilis* by inhibiting the synthesis nucleic acid [32,33]. Kazmi and coworkers (1994) defined anthraquinone as a compound isolated from *Cassia italica*, which possess bacteriostatic activity against *Pseudomonas aeruginosa, Bacillus anthracis,* and *Corynebacterium pseudodiphthericum*, and bactericidal activity against *Pseudomonas pseudomallei*. Hypericin, an anthraquinone derivative isolated from *Hypericum perforatum*, has gained much attention because of antidepressant property [34], while Duke in 1985 described that it had antimicrobial activity.

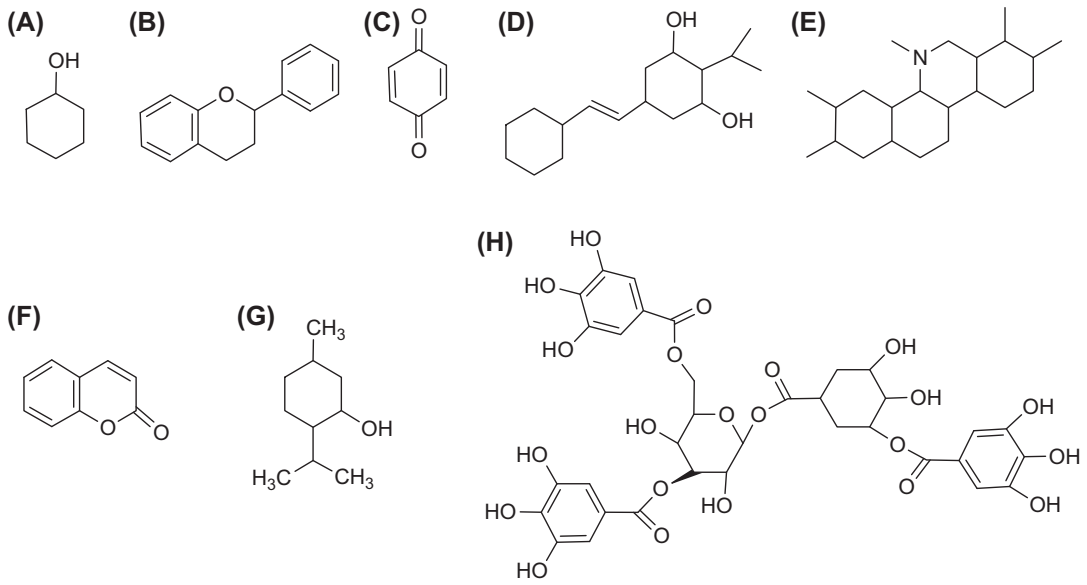


FIGURE 18.3 Structure of antimicrobial compounds from plants. (A) phenol, (B) flavonoid, (C) quinone, (D) stilbenoid, (E) alkaloid, (F) coumarin, (G) terpenoid, (H) tannin.

18.1.6.3 Stilbenoids

Stilbenoids (Fig. 18.3D) are a separate class of phenolic compounds consisting $C_6{-}C_2{-}C_6$ unit in their basic structures and are further subclassified into stilbenes, phenanthrenes, bibenzyls, oligostilbenes, and bisbibenzyls. Phenanthrenes are isolated biosynthetically from the stilbenes and bibenzyls. Stilbenes commonly occur as glycosides or aglycones, while sometimes as polymers [35]. Various families of the higher plant are supposed to synthesize stilbenes. Bibenzyls and their derivatives are rarely found in higher plants but present in some families, viz. Dioscoreaceae, Orchidaceae, and Combretaceae, which are frequently present alongside the corresponding stilbene or phenanthrene derivates. Several stilbenoids are explored for their antibacterial and antifungal attributes [36]. Eloff and coworkers (2005) have suggested that *Combretum woodii* leaves contain rich amount of bibenzyl, combretastatin B5 compounds which possess good antimicrobial activity [37].

18.1.6.4 Flavonoids

Flavonoids (Fig. 18.3B) are manufactured by herbs in response to microbial attack [38]. It has been observed that flavonoids possess antimicrobial activity against an extensive range of microorganisms during in vitro analysis, while some are showing significant activity against methicillin-resistant *Staphylococcus aureus* (MRSA) [39]. Their antimicrobial potential has been recognized by the capability to form a complex with soluble proteins, extracellular components, and the bacterial cell wall. Lipophilic flavonoids may also be responsible for microbial membranes destruction [40]. There are contradictory outcomes on the type of molecular alteration required by the flavonoid in a direction to identify antimicrobial potential. Some researcher has suggested that hydroxyl groups lacking flavonoids on their β-rings are more dynamic against microorganisms than those who comprise hydroxyl groups and this result supports the concept that microbial target is generally membrane-specific [41]. Several other workers have yet also revealed the reversible activity; the more hydroxyl groups, the greater antimicrobial activity [42]. The low toxic potential of flavonoids makes them ideal as antimicrobial medicines.

18.1.6.5 Tannins

Tannins (Fig. 18.3H) have gained attention in the last few years because of their demanding ability to treat a wide range of infections and abnormalities [43]. Tannins are again subclassified into two major classes namely proanthocyanidins (condensed tannins) and hydrolyzable tannins. Tannins have capability to form complex with proteins by nonspecific bonding, viz. hydrophobic and hydrogen bonding and also with the help of covalent binding [31]. The antimicrobial

activity of tannins may therefore be associated with their potential to denature microbial enzymes, adhesins, plasma membrane transport proteins, etc. Because of their property to bind with metals and proteins, tannins also prevent the growth of microbes through the deprivation of metal ion and substrate [27]. Hydrolyzable and condensed tannins have been suggested to show similar antibacterial and antifungal potency, although the hydrolyzable tannins seemed to be more significant against yeasts. The occurrence of a hexahydroxydiphenoyl group or its oxidatively altered entities was an essential factor for the anticryptococcal action of the ellagitannins corilagin, phyllanthusiin, and pelargoniin B [44]. The hydroxylation pattern of monomeric flavonols β-ring in condensed tannins has been found to alter the level of growth inhibition of *S. mutans* and *S. sobrinus*, *C. botulinum*, *Staphylococcus* sp., and *P. vulgaris*, and in all cases, gallocatechins were comparatively more potent inhibitor than that of their catechin counterparts [45–47]. It has also been recommended that lethality of tannin would be associated with molecular size, as larger molecule would more efficiently bind to proteins. While, in some cases the harmfulness of tannins was observed to be not greater than that of catechins [48], although catechins have a less significant binding affinity toward proteins. Kakiuchi and co-workers [49] revealed that incorporation of BSA in glucosyl transferase medium before addition of gallotannins failed to eliminate the prevention of enzyme by the tannins and they proposed that prevention of the enzyme is not essentially because of the vague binding of tannins to it. Zhu et al. [50] in their study found that some tannins suppressed ligand binding to particular receptors.

18.1.6.6 Coumarins

Coumarins (Fig. 18.3F) are also a class of phenolic compounds formed by the association of β-pyrone rings with benzene [51]. They are accountable for the specific odor of food. In *in vitro* analysis, coumarin was shown to prevent *Candida albicans*. Hydroxycinnamic acids, linked to coumarins, possess inhibitory action against Gram-negative bacteria [52]. Furthermore, phytoalexins, a hydroxylated derivative of coumarin synthesized in carrots in response to fungal contamination and can be assumed to have antifungal property [53]. The common antimicrobial property was recognized in *G. odoratum* extracts [29]. Recently, Smyth and co-workers [54] revealed the antimicrobial potential of various naturally or synthetically occurring coumarins with the help of microtitre assay against both Gram-positive and Gram-negative bacteria, along with MRSA and outcomes indicated that the coumarins were displaying good bioactivity against clinically isolated MRSA strains.

18.1.6.7 Terpenoids and essential oils

Terpenes are a major group of compounds accountable for the fragrance of herbs and encompass the essential oil fraction. They are produced from isoprenoid units and share fatty acid origins. They are vary from fatty acids in term of having cyclized and branched structure. Their common chemical formula is $C_{10}H_{16}$ and present in various forms, viz. hemiterpenes (C_5), sesquiterpenes (C_{15}), diterpenes (C_{20}), triterpenes (C_{30}), and tetraterpenes (C_{40}). When terpenes contain additional elements, mostly oxygen, then it is termed as terpenoids (Fig. 18.3G). Some common examples of terpenoids are camphor (monoterpenes), artemisinin, and farnesol (sesquiterpenoids). Terpenes and terpenoids have been shown to possess antibacterial activity [55,56], fungi [57–59], protozoa [60], and viruses [61,62]. In 1977, it was stated that 60% of the essential oil derivatives analyzed to date were prevented fungal growth, while 30% inhibits bacterial growth [63]. The molecular basis of terpenes activity is not completely known but is supposed to be involved in membrane disruption by the lipophilic compounds. Mendoza et al. [64] suggested that increasing the hydrophilicity of kaurene diterpenoids by addition of a methyl group significantly suppressed their antimicrobial activity. Cichewicz and Thorpe [65] revealed that capsaicin might trigger the growth of *C. albicans* but that it completely inhibited various bacteria to differing extents. Batista and co-workers [66] extracted two diterpenes that were known to be more democratic; they worked well against *S. aureus*, *Vibrio cholerae*, *P. aeruginosa*, and *Candida* sp.

18.1.6.8 Alkaloids

Alkaloids (Fig. 18.3E) are heterocyclic nitrogen containing a group of bioactive compounds. Morphine is the first therapeutically used natural alkaloid, extracted in the year 1805 from a flower of *Papaver somniferum*. Diterpenoid alkaloids are normally isolated from plants of the family *Ranunculaceae* [67] and are usually observed to have antimicrobial properties [68]. A glycoalkaloid namely solamargine, derived from *Solanum khasianum* berries and other alkaloids, may be beneficial in the treatment of HIV infection [69,70], intestinal disorders linked with AIDS [71]. Szlavik et al. [72] revealed that lycorine, homolycorine, and acetyllycorine hemanthamine derived from *Leucojum vernum* showed high antiretroviral actions with low medicinal indices, whereas drymaritin derived from *Drymaria diandra* had anti-HIV property [73].

18.1.6.9 Lectins and polypeptides

Peptides, which possess inhibitory property against microorganisms, were first described by Balls and co-workers [74]. Such type of peptides is positively charged and having disulfide linkage. Their mechanism of action may be governed with the ion channels in the microbial plasma membrane [75,76] or by competitive inhibition of host polysaccharide receptors by preventing microbial proteins adhesion to it [77]. Recent attentiveness has been concentrated mainly on analysis of lectins and anti-HIV peptides, but the prevention of fungi and bacteria by these macromolecules, such as that isolated from the herbaceous *Amaranthus* sp., has been properly studied [78]. Thionin peptides usually found in wheat and barley and are made up of 47 amino acid residues, reported to be lethal to yeasts, Gram-negative and Gram-positive bacteria [79]. Fabatin, a recently recognized peptide of 47 residues from fava beans, seems to be structurally similar to g-thionins from grains and prevents *P. aeruginosa*, *E. coli*, and *Enterococcus hirae* [76]. The bigger lectin molecules which comprise mannose-specific lectins are found in variety of plants [80], MAP30 from jacalin [81], and bitter melon [82] and are shown antiproliferative property in HIV and cytomegalovirus. It is possibly by inhibiting viral interaction with important components of the host cell.

18.1.6.10 Other compounds

Various phytoconstituents not described above have been shown to possess antimicrobial activity. There are a number of antimicrobial studies related to isothiocyanates [83,84], polyamines (in particular spermidine) [85], glucosides [86,87], and thiosulfinates [88]. Estevez-Braun et al. [89] identified a polyacetylene compound (C_{17}) from *Bupleurum salicifolium*, a plant native to the Canary Islands. The compound 8*S*-heptadeca-2(*Z*), 9(*Z*)-diene-4,6-diyne-1,8-diol was an inhibitor of *B. subtilis* and *S. aureus* but not to yeasts or Gram-negative bacteria. Flavonoids and acetylene compounds of plant origin have been used in Brazilian traditional system of medicine for treatment of hepatic abnormalities, and malaria fever has also been related to antimalarial activity [90]. Much has been reported about the antimicrobial potential of cranberry juice. In view of history, females have been told to drink the juice for prevention and moreover to cure urinary tract infections. In the early 1990s, researchers found that the monosaccharide fructose occurs in blueberry and cranberry juices strongly suppressed the adsorption of pathogenic *E. coli* to epithelial cells of the urinary tract, performing as an analog of mannose. Clinical analysis has disclosed the protective potential of cranberry juice. Fructose occur in numerous fruits, but researchers still seek a new bioactive compound from cranberry juice which enhances the antimicrobial activity [91,92]. Table 18.1 summarizes the major class of phytochemicals, their sources, and possible antimicrobial mechanism.

18.1.7 Recent FDA-approved antibacterial drugs

18.1.7.1 Bezlotoxumab

Bezlotoxumab (trade name Zinplava) is a completely humanized monoclonal IgG_1 antibody function against *Clostridium difficile* toxin-B [115]. Bezlotoxumab applies its activity by hindering the binding of toxin B with colonic cells and simultaneously inhibiting the growth of *C. difficile* infection [116]. In *in vitro* analysis, bezlotoxumab by deactivating the toxin B diminishes proinflammatory responses and lessens damage to epithelial tissue of colonic explants [117,118]. It has not exerted direct antimicrobial action on *C. difficile*; it has low immunogenicity and is usually well tolerated. Recent data revealed that it is also economical when administered together with standard antibiotics [119]. In October 2016, FDA approved bezlotoxumab and was obtained based on randomized, double-blind, placebo-controlled, phase III clinical trials, Modify I and II, which involved 2655 adults treated for primary or recurrent *C. difficile* infection. During trials, bezlotoxumab was linked with a smaller rate of repeated infection and had safety norms nearly the same to that of placebo [120]. An additional monoclonal antibody, actoxumab was found to act against *C. difficile* toxin A [121]. Advanced trials did not show any significant assistance in adding actoxumab with bezlotoxumab. However, the rates of continued cure were lesser compared with bezlotoxumab alone.

18.1.7.2 Ozenoxacin

Ozenoxacin approved by the FDA in December 2017 for the topical treatment of impetigo caused by *S. pyogenes* or *S. aureus* in adult and pediatric patients older than 2 months. It possess bactericidal property against vulnerable microorganisms by prevention of enzymes used in bacterial DNA replication, topoisomerase IV, and DNA gyrase A [122]. After topical application, the majority of ozenoxacin plasma samples were below the limit of quantification, suggesting no systemic absorption. Thus, the distribution, metabolism, and excretion of ozenoxacin have not been investigated

TABLE 18.1 Major classes of natural antimicrobial compounds from plants and their mechanism of action.

Class of phytochemicals	Bioactive compounds	Natural sources	Possible mechanism of action	References
Alkaloid	Berberine, Piperine	Barberry	DNA binding, inhibition CDR1, induction fungal apoptosis, intercalate cell wall	[93,94]
Carotenoid	Lycopene	Tomato	Membrane damage, induction fungal apoptosis	[95,96]
Polyphenol	Curcumin	Culinary	ROS, inhibition of morphogenetic switch and biofilm formation, membrane pore formation	[97,98]
	Resveratrol	Grape	Fungal apoptosis	[99]
Flavonoid	Amentoflavone	Selaginella	Induction fungal apoptosis	[100]
	Isoquercitirin	Starwart	Disruption of membrane	[101]
	Cathechin	Green tea	Cell wall damage	[102]
Flavones	Abyssinone		Inactive enzymes, inhibit HIV reverse transcriptase	[103,104]
Lectins and polypeptides	Mannose-specific agglutinin, fabatin	*Helichrysum aureonitens*	Block viral fusion or adsorption, form disulfide bridge	[76,105]
Quinones	Hypericin	*Hypericum perforatum*	Bind to adhesion complex in cell wall	[106,107]
Saponin	Tomatine	Tomato	Disruption of membrane, induction fungal apoptosis	[100]
Tannins	Ellagitannin	Cocoa	Binds to adhesins, binds to proteins, enzyme inhibition, substrate deprivation, membrane disruption, form complex with cell wall	[108–110]
Terpenoid	Carvacrol	Oregano	Biofilm inhibition, calcium stress	[111,112]
	Eugenol	Clove	Perturbation of cytoplasmic permeases, inhibition of ergosterol biosynthesis, interference with the integrity of the cell membrane, biofilm inhibition	[112,113]
	Caffeic acids	Tarragon	Biofilm inhibition	[114]
	Capsaicin	*Capsicum* species	Membrane disruption	[65]

in humans [123]. FDA approval was granted after a phase III randomized, double-blind, multicentre study proved the efficacy and safety of ozenoxacin in the treatment of impetigo [124]. Adverse reactions, such as rosacea or seborrheic dermatitis, were rarely reported, but prolonged use may result in overgrowth of nonsusceptible bacteria and fungi.

18.1.7.3 Ceftazidime/avibactam

Ceftazidime/avibactam is a mixture of ceftazidime, a third-generation cephalosporin that acts as a β-lactamase inhibitor in association with avibactam [125]. Ceftazidime averts formation of peptidoglycan by blocking penicillin-binding proteins, ensuing cell wall variability and cell death [126]. Avibactam and ceftazidime are eliminated through the kidney as drugs having a neutral charge. Approximately 5.7%–8.2% of avibactam and 10% of ceftazidime are bound to a protein [127]. In patients with declined renal functioning, the half-life of ceftazidime in blood is sustained and a concentration modification is suggested [128]. Antagonistic reactions that have been defined are hypersensitivity, anaphylaxis, neural functions (such as seizures), and *C. difficile*–mediated diarrhea, usually in patients with renal injury. Avibactam/ceftazidime is used for the management of typical intraabdominal disorder and intricate urinary tract problem produced by the vulnerable Gram-negative microorganisms, namely *P. aeruginosa*, *E. coli, P. mirabilis, K. oxytoca, E. cloacae, C. freundii,* and *K. pneumoniae* [129,130]. In serious intraabdominal problems, combination medication of avibactam/ceftazidime and metronidazole is suggested [131]. Avibactam/ceftazidime might be appreciated for the intensive care unit (ICU) patient while it is also used as a carbapenem-sparing antibiotic [132]. However, an analytical case series debated the rapid occurrence of resistance in medicated patients [133,134].

18.1.7.4 Vabomere

Vabomere is the mixture of a β-lactamase and carbapenem inhibitor, made up of meropenem, and vaborbactam permitted in August 2017 by the FDA for the medication of critical urinary infections in the adult [135]. Vaborbactam is an inhibitor of β-lactamase and has no antibacterial potential on its own, but it inhibits carbapenemases activity which is synthesized by *K. pneumonia* and other β-lactamases, which could cause meropenem to break down, therefore leading to analyze in vitro efficacy against *K. pneumoniae* carbapenemase Enterobacteriaceae. Meropenem susceptible microbes comprise Gram-negative bacteria such as *P. aeruginosa, E. coli*, *K. pneumoniae*, and *E. cloacae* species complex [136]. After association to penicillin-binding proteins, meropenem prevents the last step of peptidoglycan formation and hence the synthesis of cell walls without peptidoglycan, leading to lysis of bacteria. Meropenem is 2% protein bound and a half-life of 2.3 h, while vaborbactam is 33% protein bound and a half-life of 2.2 h. Furthermore, vaborbactam is not absorbed completely and is excreted through the urine in the next 2 days. Nearly 30% dose of the meropenem is utilized by β-lactam ring hydrolysis to denature form, which is passed out by the urine and between 40% and 60% amounts of meropenem is eliminated unchanged within 2 days [137]. Because of its potential against multidrug-resistant microbes, vaborbactam-meropenem displays promising suggestions in the treatment of ventilator-mediated pneumonia [138,139]. Any of the two constituents or other drugs in the same class is admonition in patients suffering from hypersensitivity and the most commonly faced adversative reactions, viz. phlebitis or infusion site reactions, headache, and diarrhea [135]. Rare but severe side effects comprise hypersensitivity reactions, thrombocytopenia, seizures, *C. difficile*—mediated diarrhea, occurrence of drug-resistant bacteria, neuromotor impairment, and excessive growth of nonvulnerable microbes [136]. Doses should be modified and precaution must be taken by the patients with compromised renal function [140].

18.1.7.5 Ceftaroline

Ceftaroline is a new broad-spectrum antibiotic used for the treatment of penicillin- and cephalosporin-resistant *S. pneumoniae,* methicillin-resistant *Staphylococcus aureus*, vancomycin-resistant *S. aureus*, and vancomycin-intermediate *S. aureus* [141]. It also shows activity against several Gram-negative microbes but nonfunctional against β-lactamase synthesizing bacteria. It has been widely accepted for the medication of pneumonia. Ceftaroline was prepared by an advance alteration in the structure of fourth-generation cephalosporin cefozopran [142]. This drug shows activity by forming an association with penicillin-binding proteins. It also possesses much affinity toward *S. aureus* penicillin-binding protein-2a (PBP2a), which is responsible for methicillin resistance. Other than the blood circulation, ceftaroline fosamil (prodrug) is quickly transformed to ceftaroline (an active form) with the help of phosphatase enzymes. It displays linear pharmacokinetics and has a half-life (t½) of 120 min (for a single dose) to 155 min (for multiple doses) in serum. Ceftaroline is processed by hydrolysis of its β-lactam ring, which further leads to the formation of neutral, open-ring metabolite termed as ceftaroline M-1. It has less potential for drug interactions because of inappropriate breakdown by CYP450 enzymes [143]. Dose regulation is mandatory in patients with renal injury as it is predominantly excreted by the kidney. The most common adverse effects detected in clinical trials were headache, dysgeusia, vomiting, diarrhea, and nausea.

18.1.7.6 Ceftobiprole

It is a newer form of cephalosporin which has accomplished its trial in 2007 and is waiting for approval by the FDA because of supplementary safety documents being required by the FDA. Ceftobiprole is a broad-spectrum antibiotic which possess a wide range of activity against *Enterococci,* MRSA, penicillin-resistant *P. aeruginosa*, and *S. pneumoniae* [144]. It exhibits high affinity for PBP2x of *S. pneumoniae* and PBP2a of MRSA [145]. It is given as IV infusion in 1 h every 12 h for Gram-positive infection and a 2-hr infusion of 500 mg every 8 h for Gram-negative infection. Dose adjustment is needed in patients with renal impairment [146]. It is well-tolerated with the most usual side effects being nausea and dysgeusia.

18.1.7.7 Doripenem

This drug is permitted for the medication of complex intraabdominal infections or urinary tract infections (UTI). It performs its function by binding with PBPs and consequently averting the cross-linking structure of the peptidoglycan. The high binding affinity of doripenem with PBP-2 and -3 may boost its potential against drug-resistant *P. aeruginosa*. Therefore, it is an appropriate option to presently used antipseudomonal carbapenems (i.e., meropenem, imipenem) [147]. It demonstrates activity against Gram-positive cocci and Gram-negative bacilli such as imipenem and meropenem, respectively [148]. Doripenem, in similarities to other carbapenems, is stable to ESBLs synthesized by *E. coli* and Klebsiella species and to AmpC β-lactamases enzymes; but it is susceptible to some acquired β-lactamases such as class B

metallo-β-lactamases produced by some *P. aeruginosa* isolates and carbapenemases produced by some *Enterobacteriaceae* and *Acinetobacter* species [149]. 500 mg in every 8 h through IV is a recommended dose of doripenem. Protein binding is low (8.1%) and is independent of the amount of drug in the plasma. The projected excretion half-life of doripenem is 57 min, and 75% of the drug is excreted as an uncharged molecule through the urine; recommended dosage modifies in renal damage patients [150]. The most common ADRs are nausea, phlebitis, diarrhea, and headache and rash. Postmarketing reports have also recognized toxic epidermal necrolysis, Stevens−Johnson syndrome, seizures, and interstitial pneumonia as antagonistic drug reactions.

18.1.7.8 Telithromycin

It is the first ketolide to enter clinical use for the treatment of CAP, chronic bronchitis, and acute sinusitis. Other ketolides are in the phase of clinical development. It acts as an inhibitor of protein synthesis that prevents the advancement of the growing polypeptide chain by binding to lager subunit (50S) of the microbial ribosome. It shows 10-folds more affinity to the larger subunit of ribosome in comparison with erythromycin. Furthermore, telithromycin simultaneously binds strongly with two domains of 23-S RNA of larger subunit of the ribosome, while older macrolides bind strongly only with one domain and loosely with the second domain. In *in vitro* analysis, it is found to be more effective against *S. pneumonia* followed by clarithromycin and azithromycin and retains their property against macrolide-resistant strains (*S. pyogenes, S. pneumoniae*) [151]. Telithromycin has marketed as a tablet form with a concentration of 400 mg for oral administration. It is well metabolized orally with 60% bioavailability. A terminal half-life of 9.81 h is attained with a single 800 mg daily oral dose with peak plasma concentration of 2.27 mg/L [152]. It achieves high tissue concentrations in respiratory fluids and tissues, including epithelial lining fluid, alveolar macrophages, bronchial mucosa, and saliva [153]. Telithromycin is 66%−89% bound to serum protein, mainly albumin. The drug is excreted predominantly by hepatic metabolism, 50% by CYP3A4, and 50% by CYP-independent metabolism. No dose modification is required for patients with hepatic failure or mild to moderate renal abnormalities.

18.1.8 Antibacterial drugs on clinical trial

No doubt herbs and their bioactive compounds have been serving for centuries to protect living beings from a wide range of infections and illness but organized clinical trials are scarce. In some of the cases, traditional therapists together with an expert scientist working in the area of keeping records of the efficacy of the phytochemicals medication and safety measurements; however, these are usually randomized and unplanned studies. Some of the clinical trials associated with the use of phytochemicals in the welfare of humans are briefly summarized here.

In West Africa, to reveal the efficacy of toothbrushes versus chewing sticks by cross-sectional epidemiological study in encouraging oral hygiene, a study was conducted. Research associated with this study found a lowered effectiveness in chewing stick in comparison with toothbrush and suggested that chemicals responsible for antimicrobial activity are known to occur in the sticks not promoted oral health benefits [154]. Furthermore, mouth washes containing various antimicrobial compounds have been examined in humans [155]. Mouthwash having phytoconstituents were not found to be as potent in suppressing plaque or gingivitis as was chlorhexidine or Listerine. Till now, few unsystematic clinical trials of herbal antimicrobial agents have been executed. Giron et al. [156] compared *Solanum nigrescens* extract with nystatin, both given as intravaginal suppositories in female with confirmed *C. albicans* vaginitis. The extract supposed to be as effective as nystatin. In 1996, cranberry juice was tested against urinary tract infections in a group of aged women. The researcher did not reveal any data related to this clinical study, whereas medicated women had fewer microbes in their urine than did the untreated group [92]. Four different formulations of natural compounds as reported in Ayurveda were examined against a placebo for their action against acne vulgaris. One of the formulations namely Sunder Vati produced an appreciably greater drop in lesion count than did the other three as well as the placebo. No adverse side effects were noticed [157]. Two proprietary compounds isolated from tropical plants, provir, for the management of viral infections associated with respiration, and virend in 1994, a topical antiherpes compound were evaluated in clinical trials [158]. Meanwhile then, the efficacy and safety of virend have been established in phase II studies [159]. More information on plants and phytochemicals with antiviral properties can be found on chapter 15 of this book.

18.1.9 Future perspectives and conclusion

The need for effective curative therapies is constantly on the increase because of antibiotic-resistant microbial pathogens. The continuing scarcity of strong antibiotics is neither just a biological issue (evolution in resistance) nor even a public problem (misapplication of antibiotics and selection of resistance), although is in broader side the consequence of a

long-term gap of investigation in research and advancement programs by most of the pharmaceutical industries. Nowadays, there are a very small fraction of antibiotics in late phase of clinical trials, and practically all of these are belonging to preexisting classes. The causes for this investment break in novel classes are multifaceted, but they are linked with the least assurance on the part of the pharmaceutical companies in the comparative profitability of antimicrobials as a good option for investment. As clinical trials demonstrate the bulk of the expenditure of drug advancement, it is preferable to remove strongly problematic molecules in the early hit-to-lead or lead-to candidate phases, rather than have an expensive failure. The high rate of attrition in antibiotic drug development makes it essential, and hence that investment in early stage of research and advancement is continued at a high enough level to produce a sustainable pipeline of novel ideas used into advance stages of development and serving into clinical trials.

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