CHAPTER - V

ANTI-MICROBIAL STUDIES

Coordination complexes of transition metal had been widely studied for their antimicrobial\textsuperscript{[1,2]} and anticancer properties.\textsuperscript{[3,8]} One of the most potent and effective antitumour agents was discovered in the last century serendipitously by Rosenberg\textsuperscript{[9]}. Rosenberg and his coworkers synthesized several simple platinum 3 2 2 complexes, among which cisplatin - Pt(II)(NH)\textsubscript{2}Cl\textsubscript{2} - showed remarkable efficacy in inhibiting the growth of tumours in mice\textsuperscript{[10]}. McGowan\textsuperscript{[11]} reported the first clinical trials of cisplatin in 1971, with official approval being granted in the US in 1978. Despite the success of cisplatin, however, it lacks selectivity for tumour tissue, which leads to severe side effects including renal impairment, neurotoxicity and ototoxicity. Various tumor cell lines are now growing resistance to cisplatin e.g., acquired cisplatin resistance in some preclinical tumor models\textsuperscript{[12]}. The scientists are now engaged to explore other transition metal complexes as antitumour agents and considerable results have brought through the discovery of titanium based complexes\textsuperscript{[13,14]} and other transition metal based complexes\textsuperscript{[15,21]}. Among the other transition metal complexes the titanium complex, titanocene 2 2 dichloride (TiCp\textsubscript{2}Cl\textsubscript{2}) is the only metallocene-based compound to have entered clinical trials for its potent and broad spectrum activity in mammalian tumors\textsuperscript{[11]}. Compared to standard antineoplastic agents such as cisplatin, doxorubicin, mitoxantrone and vinblastine, titanocenedichloride was found to exhibit higher cytotoxicity in renal cell carcinoma\textsuperscript{[13]}. The titanocenedichloride was found to exhibit
more effective in human ovarian cancer xenograft model than cisplatin\textsuperscript{[14]}. Recently some derivatives of titanocenedichlorides showed enhanced anti-cancer activity\textsuperscript{[22]}. Therefore, it is of our interest to study the cytotoxic and antimicrobial properties of some novel coordination complexes of different transition metals to assess their biological potency. We have found promising cytotoxic and antimicrobial activities of these novel complexes and further studies on mammalian cancer cell lines may explore their valuable cytotoxicity which may come as potent anticancer agent(s) in the modern clinical trials.

Post-operative infections may be overcome by adjusting antimicrobial properties of the implant surface prior to implantation. Techniques described in literature are direct impregnation with antibiotics \textsuperscript{[8]} prior to implantation or using antibiotic or silver doped polymer coatings. Silver-based antimicrobials are of interest due to the non-toxicity of the active Ag\textsuperscript{+} to human cells \textsuperscript{[9,10]} and the antimicrobial activity of silver ions has been well established. Silver ions are significant antimicrobials with only few bacteria being intrinsically resistant to this metal through plasmid derived resistance mechanisms\textsuperscript{[11-14]}. Incorporation of silver ions into polymeric materials has been widely used for several years; especially urinary and central venous catheters are provided with silver coatings to reduce infections. Medical devices like heart valves or dialysis units also benefit from the use of silver doted surfaces \textsuperscript{[15-18]}.

However, most of the techniques used do not fulfil the mechanical criteria for load-bearing implants because especially the implantation
into the bone results in high (abrasive) shear forces between bone and the implant surface. The aim of this study was the development of a metallic hard coating based on titanium/silver alloys which provides antimicrobial properties due to the silver content as well as a high biocompatibility in contact with bone. Titanium samples were coated with the silver-titanium-alloys containing up to 9% silver by using the physical vapour deposition (PVD) process and tested for hardness, biocompatibility and bactericide action. The PVD process is known to result in good adhesiveness and wear resistance of metallic or ceramic coatings and is widely used in technical and medical applications [19]. The coatings are aimed to provide antimicrobial properties due to the release of silver ions in an aqueous environment while maintaining the biocompatibility and hardness of titanium against hard and soft tissue for an application on load-bearing implants, e.g. in hip or knee arthroplasty.

Non-ribosomally synthesized peptides have compelling biological activities ranging from antimicrobial to immunosuppressive and from cytostatic to antitumor. The broad spectrum of applications in modern medicine is reflected in the great structural diversity of these natural products. They contain unique building blocks, such as D-amino acids, fatty acids, sugar moieties, and heterocyclic elements, as well as halogenated, methylated, and formylated residues. In the past decades, significant progress has been made toward the understanding of the biosynthesis of these secondary metabolites by nonribosomal peptide synthetases (NRPSs) and their associated tailoring enzymes. Guided by this knowledge, researchers genetically redesigned the NRPS template to synthesize new peptide products. Moreover, chemoenzymatic strategies
were developed to rationally engineer nonribosomal peptides products in order to increase or alter their bioactivities. Specifically, chemical synthesis combined with peptide cyclization mediated by nonribosomal thioesterase domains enabled the synthesis of glycosylated cyclopeptides, inhibitors of integrin receptors, peptide/polyketide hybrids, lipopeptide antibiotics, and streptogramin B antibiotics.

Natural products that are produced by microorganisms have for decades attracted considerable attention for modern therapy. The bioactivity of these structurally complex substances extends from antibiotic to immunosuppressive and from cytostatic to antitumor (106). Not only have these secondary metabolites been optimized for their dedicated function over millions of years of evolution, they also represent promising scaffolds for the development of novel drugs with improved or altered activities. Optimization can be achieved by the introduction of artificial modifications, which yields semisynthetic derivatives of existing structures, although total synthesis of complete natural-product-based compounds is also envisioned.

Peptidic products represent a large subclass of highly diverse natural products, many of which display therapeutically useful activity. They can be classified into different groups according to their synthesis pathways. The lantibiotics, for example, are ribosomally synthesized antimicrobial agents that are posttranslationally modified to their biologically active forms. Yet another class, a widespread class of therapeutically important peptides, are produced nonribosomally by large multienzyme complexes, the nonribosomal peptide synthetases
(NRPS). In contrast to ribosomal peptide synthesis, non-ribosomally assembled peptides contain not only the common 20 amino acids (aa) but hundreds of different building blocks. Moreover, these secondary metabolite peptides contain unique structural features, such as D-amino acids, N-terminally attached fatty acid chains, N- and C-methylated residues, N-formylated residues, heterocyclic elements, and glycosylated amino acids, as well as phosphorylated residues. In recent research using both genetic and biochemical methods, experiments have revealed deep insights into the mechanism of nonribosomal peptide synthesis. In many cases, it was possible to alter existing non-ribosomally produced peptides by the combined action of chemical peptide synthesis and subsequent enzyme catalysis. This chemoenzymatic approach, along with a brief overview of the nonribosomal peptide synthesis machinery, will be discussed in more detail later in this review. Another focus of this article will be the labeling of NRPS-derived proteins by site-specific posttranslational modification. Selected structures of some non-ribosomally produced peptides are shown in Fig. 1. A common feature of these compounds is their constrained structure, which ensures bioactivity by a precise orientation required for interaction with a dedicated molecular target (68). In some cases, these constraints are imposed by heterocyclization. For instance, the iron-chelating siderophore vibriobactin comprises two oxazoline rings, both of which originate from threonine residues. This oxazoline ring can be further oxidized to yield oxazole, as found in the potent telomerase inhibitor telomestatin. In addition to oxazoles, telomestatin also contains a thiazoline ring that is synthesized by the heterocyclization of cysteine. In the case of the antibiotic bacitracin, this heterocyclic element mediates a specific cation-
dependent complexation of the phosphate group of the C₅₅ lipid carrier, leading to depletion of this carrier and subsequent blocking of bacterial cell wall synthesis. An additional strategy to modify and thus constrain the conformation of nonribosomal peptides is exemplified by the glycopeptide antibiotics of the vancomycin and teicoplanin class. These closely related compounds contain a homologous heptapeptide scaffold, whose backbone is constrained by extensive oxidative cross-linking. The joining of electron-rich aromatic rings by aryl ether linkages and direct C-C coupling convert these acyclic, floppy heptapeptides into rigid, cup-shaped structures. The constrained glycopeptides sequester the N-acyl-D-Ala-D-Ala termini of bacterial peptidoglycan strands with five hydrogen bonds and inhibit the transglycosylation and/or transpeptidation steps of bacterial peptidoglycan synthesis.

Macrocyclization is another common constraint of non-ribosomally synthesized peptides whereby parts of the molecule distant in the linear peptide precursor are covalently linked to one another. Thus far, many biological strategies for the cyclization of nonribosomal cyclopeptides have been identified, giving rise to a high diversity in this class of compounds. For instance, the intramolecular capture by amines leads to peptidolactams, whereas cyclization via hydroxyl substituents leads to peptidolactones. The former strategy is observed for the peptide antibiotics tyrocidine A, bacitracin, gramicidin S, and the immunosuppressive drug cyclosporine. In the case of tyrocidine A, amide bond formation occurs head-to-tail between the N-terminal amino group and the C terminus of the decapeptide. An unusual type of head-to-tail cyclization is observed for nostocyclopeptide, where the terminal ends of
the peptide are linked via an imine bond. In contrast, the dodecapeptide bacitracin has a lariat structure, with the heptapeptide lactam ring arising from capture of the C-terminal carbonyl group by the \( \varepsilon \)-amino group of Lys6. Moreover, the macrolactam gramicidin S is composed of two identical pentapeptides bridged head-to-tail yielding a symmetric dilactam ring. For macrolactones, analogous cyclization strategies lead to branched-cyclic structures as seen for the antifungal lipopeptide fengycin, the antibiotic pristinamycin, and the biosurfactant surfactin A. The former depsipeptides are cyclized via the side chains of hydroxy amino acids such as tyrosine and threonine, whereas the latter compound is cyclized via a \( \beta \)-hydroxylated fatty acid moiety. Finally, the iron-chelating siderophore bacillibactin is a cyclic trilactone that arises from cyclotrimerization of threonine.

The structural diversity of non-ribosomally produced peptides is best exemplified for the class of acidic lipopeptide antibiotics, including the calcium-dependent antibiotic (CDA) from Streptomyces coelicolor, daptomycin from Streptomyces roseosporus and A54145 from Streptomyces fradiae, as well as friulimicins and amphomycins from Actinoplanes friuliensis. All of these lipopeptides originate from streptomycetes, which produce over two-thirds of naturally derived antibiotics. Each member of this class of lipopeptides can be subdivided into various individual compounds that differ in the structure of the N-terminally attached fatty acid moiety and/or the peptide backbone (Fig. 2). For example, A54145 is a complex of eight lipopeptides which are acylated with an 8-methylnonanoyl, \( n \)-decanoyl, or 8-methyldecanoyl lipid side chain. These factors also contain four different cyclic peptide
nuclei which differ in glutamate/3-methylglutamate (position 12) and/or valine/isoleucine (position 13) substitutions. The diversity of acidic lipopeptide antibiotics is further amplified by the occurrence of D-configured as well as nonproteinogenic amino acids, including D-4-hydroxyphenylglycine (D-HPG), D-3-phosphohydroxyasparagine, 3-methylglutamate (3mGlu), D-pipecolic acid, kynurenine (Kyn), and many more. Interestingly, all of the acidic lipopeptide antibiotics are comprised of a branched cyclic decapetide lactone ring or lactam ring. The positions of the D-configured amino acids are strictly conserved in this macrocyclic scaffold. Moreover, two aspartic acid residues are found in equivalent ring positions of the macrolactone or macrolactam ring. Recently, a genomics-based approach revealed the existence of numerous uncharacterized lipopeptide biosynthetic gene clusters, indicating that many more antibiotics of this class have yet to be identified.

The therapeutic importance of the acidic lipopeptide antibiotics is best exemplified for daptomycin. This amphiphatic tridecapeptide is a member of the A21978C complex produced by S. roseosporus (Fig. 2). Although the major components, A21978C₁ through A21978C₃, have 11-, 12-, or 13-carbon fatty acids, the yield of daptomycin (10-carbon fatty acid) from fermentations is significantly increased by adding decanoic acid to the medium. Daptomycin (Cubicin; Cubist Pharmaceuticals), exhibits bactericidal activity against resistant pathogens for which there are very few therapeutic alternatives, such as vancomycin-resistant enterococci, methicillin-resistant Staphylococcus aureus, and penicillin-resistant Streptococcus pneumoniae. At present, spontaneous acquisition
of resistance to daptomycin is rare, which might be due to a unique mechanism of action.

Although the mechanism of action of daptomycin is not yet fully understood, it has been clearly established that calcium ions play an essential role in antimicrobial potency. A nuclear magnetic resonance (NMR) study indicated that the stoichiometry of Ca$^{2+}$ binding to daptomycin is one to one (2). Therefore, the total charge of the Ca$^{2+}$-conjugated daptomycin (−1) is lower than that of Ca$^{2+}$-free daptomycin (−3) at a neutral pH. This would result in a more hydrophobic molecule due to charge neutralization, facilitating interaction of Ca$^{2+}$-conjugated daptomycin with lipid bilayers. It has been proposed that, upon association with bacterial cytoplasmatic membranes, a major Ca$^{2+}$-dependent conformational change promotes deeper insertion of daptomycin into the lipid bilayer. This is followed by large membrane perturbations, including lipid flip-flop and membrane leakage. Formation of any of these presumably disrupts the functional integrity of the membrane leading to cell death of gram-positive bacteria.

Although some of the key structural prerequisites for daptomycin’s antibacterial activity have been identified, the exact nature of the molecular targets within the cytoplasmatic membrane has yet to be established. However, the aforementioned model of the mechanism of action provides an initial step toward understanding how this antibiotic gains access to and interacts with bacterial membranes. Since the other acidic lipopeptide antibiotics CDA, A54145, friulimicins, and amphomycins share key structural features with daptomycin; they might
undergo similar interactions with calcium ions and bacterial membranes. Therefore, it is essential to further probe the structure-function relationship of all acidic lipopeptide antibiotics. Using this knowledge will enable the design of new and improved derivatives of this remarkable class of antibiotics. However, in order to engineer more potent variants, one has to understand the biosynthesis of these complex compounds.

Despite the structural diversity of the non-ribosomally produced acidic lipopeptide antibiotics, these secondary metabolites share a common mode of synthesis, the so-called "multiple carrier thio-template mechanism". According to this model, peptide synthesis is performed by nonribosomal peptide synthetases (NRPSs). Figure 3 shows the NRPS assembly lines for daptomycin, A54145 and CDA. Detailed analysis of the daptomycin gene cluster revealed that the daptomycin biosynthetic system consists of three distinct NRPSs, namely, DptA (684 kDa), DptBC (815 kDa), and DptD (265 kDa). In contrast, the closely related A54145 biosynthetic system comprises four NRPSs (LptA, LptB, LptC, and LptD). It is assumed that DptBC arises from a fusion of two NRPSs similar to LptB and LptC. Finally, the nonribosomal CDA biosynthetic system is a multienzyme complex consisting of three enzymatic subunits, CdaPS1 (799 kDa), CdaPS2 (395 kDa), and CdaPS3 (259 kDa).

The multifunctional NRPSs of daptomycin, A54145, and CDA are organized into sets of repetitive catalytic units called modules (Fig. 3). Each module is responsible for the specific incorporation of one residue into the peptide backbone. Therefore, the number of modules within the NRPSs exactly matches the number of residues of the corresponding
peptides. Moreover, the order of modules corresponds directly to the primary sequence, because nonribosomal peptide synthesis proceeds colinearly in an N-terminal-to-C-terminal direction. Such biosynthetic templates are also referred to as linear NRPSs (type A). In contrast to that, iterative NRPSs (type B) use their modules or domains more than once in the assembly of peptides that consist of repeated smaller sequences. Finally, nonlinear NRPSs (type C) constitute a considerable fraction of the NRPS repertoire where the sequence of the product does not directly correspond to the linear arrangement of modules and domains within the biosynthetic template. These various biosynthetic strategies of nonribosomal peptide synthesis were extensively reviewed by Mootz et al.

The proper coordination of communication between partner NRPSs in trans (i.e., the last module of DptA and first module of DptBC) is facilitated by short regions at the C and N termini of the corresponding proteins. These communication-mediating (COM) domains, also referred to as docking domains, comprise 15 to 30 amino acid residues and prevent undesired interactions between mismatching NRPSs (i.e., the last module of DptA and first module of DptD), which would lead to the formation of truncated peptide products. Sequence alignments revealed that the overall identity among COM domains is low, reflecting the high degree of specialization for their dedicated partner COM domains. The first structural insights into the interaction between multimodular subunits were gained from NMR spectroscopy on related polyketide synthases (PKS). Studies of fused docking domains of the 6-deoxyerythronolide B synthase (DEBS) multienzyme subunits DEBS2 and
DEBS3 revealed that protein-protein recognition is primarily mediated by interhelical contacts. The most important determinant of docking is a set of conserved hydrophobic interactions between four α-helices, which together form the core of a parallel four-helix bundle. In addition to the hydrophobic interface, two partially buried salt bridges between two of these α-helices may play a role in stabilizing this docking interaction. Furthermore, such ionic contacts might contribute to the destabilization of misdocked partner PKS subunits. Knowledge of the structural aspects of intersubunit communication may contribute to engineering of optimized protein-protein interfaces between NRPS, PKS, and mixed NRPS/PKS systems.

NRPS modules are further subdivided into domains that catalyze the single reaction steps, such as amino acid activation, covalent binding of activated residues, amide bond formation, epimerization of covalently bound residues, and peptide release from the NRPS complex.

Binding of three macrocyclic bis-intercalators, derivatives of acridine and naphthalene, and two acyclic model compounds to mismatch-containing and matched duplex oligodeoxynucleotides was analyzed by thermal denaturation experiments, electrospray ionization mass spectrometry studies (ESI-MS) and fluorescent intercalator displacement (FID) titrations. The macrocyclic bis-intercalators bind to duplexes containing mismatched thymine bases with high selectivity over the fully matched ones, whereas the acyclic model compounds are much less selective and strongly bind to the matched DNA. Moreover, the results from thermal denaturation experiments are in very good
agreement with the binding affinities obtained by ESI-MS and FID measurements. The FID results also demonstrate that the macrocyclic naphthalene derivative BisNP preferentially binds to pyrimidine–pyrimidine mismatches compared to all other possible base mismatches. This ligand also efficiently competes with a DNA enzyme (M.TaqI) for binding to a duplex with a TT-mismatch, as shown by competitive fluorescence titrations. Altogether, our results demonstrate that macrocyclic distance-constrained bis-intercalators are efficient and selective mismatch-binding ligands that can interfere with mismatch-binding enzymes.

Mismatched base pairs in DNA can arise by several processes. One of their most important sources are the replication errors, i.e. direct misincorporation of bases due to statistical errors during DNA replication, or due to damages, or lesions, in the parental strand, which lead to incorrect recognition and thus incorporation of wrong bases in the newly replicated strand.\textsuperscript{1-3} Another mechanism is based on the formation of a heteroduplex between two homologous DNA molecules during the recombination process: if the two DNA strands differ slightly in their sequence, mismatched base pairs may be formed.\textsuperscript{4} Mismatches may also be generated in hairpins that are formed between imperfect palindromes existing in repeat-containing sequences, such as microsatellites and trinucleotide repeats.\textsuperscript{5,6} An additional special but important path for the formation of mismatched base pairs is deamination of 5-methylcytosine, a modified base that is present in the DNA of many organisms. In this case, the deamination product is thymine and a GT mismatch is generated.\textsuperscript{7}
Base mispairs can be hazardous to the cell in altering its ability to transfer the information content of DNA. In particular, mismatches may result in point mutations that are potentially harmful, depending on where they occur in the genome. Consequently, every organism has evolved a variety of control and repair strategies based on complex enzymatic machineries responsible for the maintenance of DNA integrity. In particular, mismatches are recognized and repaired by specific enzymes, which constitute the mismatch repair (MMR) system. The MMR system is based on a complex network of interactions between various enzymes and is closely related to the other systems recognizing and signaling lesions (base-excision repair, nucleotide-excision repair, methyl-directed repair, etc.).

In addition, it is well established that most human tumors develop through a succession of genetic and epigenetic changes and that high frequency of mutations is clearly correlated to cancerogenesis. For instance, mutations of oncogenes or tumor suppressor genes, such as p53 and BRCA, and sequence instability of microsatellites are directly related to the occurrence of inherited cancers. Finally, the importance of DNA repair in the prevention of carcinogenesis has been recently highlighted by finding a direct correlation between the defective DNA MMR and hereditary colon cancer.

In terms of molecular recognition, repair of DNA mismatches requires that the correct base in the mispair is distinguished from the incorrect one. Since both bases in a mispair are regular constituents of DNA, this recognition is much more challenging compared with the recognition of damaged bases, which are structurally altered compared
with the common DNA bases. For instance, the specific recognition of GT mispairs by the MutS enzyme is fascinating, because they are thermodynamically only slightly less stable than the Watson–Crick base pairs\textsuperscript{13} and induce only a slight structural modification of duplex DNA\textsuperscript{14–18}. Therefore, studies aimed at a deeper understanding of the recognition of mismatches by repair enzymes have raised continued attention for more than a decade. Several models have been proposed to rationalize the mechanisms of mismatch recognition, but these are still poorly understood.\textsuperscript{14} Given the complexity of these processes the task is highly challenging and requires several approaches, such as genetic, biochemical and chemical ones. In particular, a chemical tool for studying mismatch recognition is represented by small molecules that, similar to the mismatch-recognizing enzymes, can bind base mispairs with a high selectivity over fully paired DNA. Such mismatch-binding ligands (mismatch binders) may eventually interfere with the repair systems with negative or positive consequences, leading to inhibition\textsuperscript{19} or promotion of repair, and thus display high therapeutic potential.

DNA mismatches constitute sites of reduced thermodynamic stability compared with Watson–Crick base pairs\textsuperscript{20–22}, which leads to enhanced conformational dynamics (‘breathing’) and may facilitate the insertion of classical intercalators and various drugs into these sites.\textsuperscript{23–25} The design of mismatch binders usually takes advantage of this local conformational suppleness, although it is only modest, restricted to two to three adjacent base pairs and depends on the type of mismatch. Importantly, beyond the specific mismatch recognition motifs efficient mismatch binders should feature unfavorable interactions with Watson–
Crick base pairs as well as with grooves, to ensure low binding to the fully paired DNA and high selectivity for the mismatches. Thus, in the past decade several series of mismatch-recognizing agents have emerged. Among these are molecular systems that operate via intercalation, such as rhodium-based metalloinsertors, which preferentially bind to CC and CA mismatches, or via bis-intercalation, such as bis-naphthyridine derivatives that selectively bind to GG and GA mismatches. Minor groove binders such as imidazole-rich polyamides have also been shown to selectively bind to the GT mismatched sites, recognizing the geometric modifications of the grooves induced by this mispairing. In another approach, we have shown that a macrocyclic bis-acridine compound (BisA, Chart 1) recognizes base-pairing defects, like abasic sites and thymine-containing mismatches, such as TT, TC and, to a lesser extent, TG-mismatched base pairs, via a putative threading bis-intercalation mode. Moreover, insertion of BisA at TX-mismatch sites induces a displacement of the thymine into an extrahelical position, as shown by the susceptibility of the flipped-out thymine to oxidation by KMnO₄. This established for the first time that base flipping, which is characteristic of DNA methyltransferases and DNA glycosylases, can also be performed by a small molecule. Subsequently, several studies have shown that other mismatch binders, such as naphthyridine dimers and bulky rhodium-containing metalloinsertors are also able to displace bases into extrahelical positions.

Beyond their potential usefulness for interfering with the MMR system, the mismatch binders may also serve in genetic diagnostics for the detection of point mutations and single-nucleotide polymorphism
Towards this objective, several mismatch-binding ligands have been endowed with cross-linking\textsuperscript{45}, photoactive\textsuperscript{46,47} or fluorescent groups\textsuperscript{48}. Such molecular probes could complement the number of chemical, physical and enzymatic methods\textsuperscript{49-51} developed for mismatch detection since most exhibit drawbacks and/or questionable reliability.\textsuperscript{52}

In order to get deeper insight into the interaction of macrocyclic compounds, such as \textbf{BisA}, with mismatches, we carried out a systematic study aimed at the determination of structural factors that determine the binding, as well as the stoichiometric and thermodynamic parameters of the binding event. To achieve this goal, we extended the macrocyclic series by several analogues of \textbf{BisA} and studied their mismatch-binding properties by a number of biochemical and spectroscopic methods. First, we prepared a derivative with amine-terminated side chains in the linkers between the two acridine units (\textbf{BisA-NH}_2, \textit{Chart 1}), which provides a higher cationic charge as well as possibility for further functionalization.

Second, to evaluate the importance of stacking interactions the acridine units were replaced by smaller naphthalene moieties (\textbf{BisNP}). Finally, we prepared the control compounds \textbf{DMA1}, containing two acridine units linked in an acyclic framework, and \textbf{MonoNP}, containing one naphthalene unit endowed with two side chains identical to the linkers used in \textbf{BisNP}. In the current work, we report the binding properties of this novel series of ligands towards thymine-containing mismatched DNA duplexes and their fully matched counterparts, studied with a set of biophysical methods, i.e. thermal denaturation, mass spectrometry and fluorescent intercalator displacement (FID) experiments. We show that the most efficient ligand of the series, namely \textbf{BisNP}, has strong
preference for pyrimidine–pyrimidine mismatches, binds them with nanomolar affinity and is able to significantly interfere with binding of the DNA methyltransferase M.TaqI, which binds to a TT mismatch in its recognition sequence.

Obviously zinc(II) complexes were more reactive than the free ligands. This might be due to that the cation density in increased with the existence of zinc(II), it was more available for complexes to bind DNA with anion.

To investigate whether the macro cyclic polyamine imidazolium salt ligands and their zinc (II) complexes were efficient reagents in protecting DNA from being cleaved enzymatically, 0.1 U of DNasel was added respectively to the plasmid DNA complexes containing 7 µg/ml of DNA. The result were shown in Figure 3. DNasel could cleave DNA completely (Lane 2), which was used as control. It is obvious that both ligand 6 and complexes 1 could retard the cleavage of DNA efficiently (Lanes 3-6). It was illustrated that DNA could be protected by those compounds to avoid being cleaved. There might be a few possible reasons for the DNA protection based on imidazolium salt. The first one is that the positive charge on the imidazolium group kept Mg$^{2+}$ away from the positively charged imidazolium salt. This would retard the enzymatic cleavage process, in which Mg$^{2+}$ is needed. The second one is that DNA surface binding with imidazolium salt resulted in a variation of the DNA structure due to the size effect.

A typical imidazolium 1-ethyl-3–methylimidazolium bromide (EMI) can not bind DNA. Clearly, macropolyamine moiety takes a
significant role when ligands or complexes interact with DNA. Polyamine could be protonated then possesses more cation density to bind DNA with anion, maybe so ligands 6 and complexes 1 are more active than imidazolium without macropolyamine moiety.

With the discovery of penicillin as an efficient antibacterial agent isolated from the fungus Penicillium notatum, microorganisms attracted considerable attention as a new source for pharmaceutical agents. Screening of microbial extracts revealed the large structural diversity of natural compounds with broad biological activities, such as antimicrobial, antiviral, immunosuppressive, and antitumor activities. Like penicillin, many of these products are small peptide molecules consisting of 3 to 22 residues with often unusual structural elements. These include heterocyclic elements, D-amino acids, and glycosylated and N-methylated residues, suggesting a nonribosomal origin of biosynthesis.

Due to the potent pharmacological activities of these compounds, there was an overwhelming interest in exploring their mechanism of synthesis. Lipmann et al. reported as early as the 1970s that the cyclic peptides gramicidin S and tyrocidine from Bacillus spp. were produced in a nucleic acid-independent way through the use of large enzyme complexes similar to fatty acid synthases. Subsequently, other peptidic natural products were shown to be assembled by large enzymes, referred to as nonribosomal peptide synthetases (NRPS), which utilize the multiple-carrier thiotemplate mechanism. A common feature of many nonribosomally produced peptides is their constrained structure, which ensures a precise functionality important for a proper interaction with the dedicated molecular target in the cell. Nature achieves this rigidity in molecular structure through several strategies: the molecule can be
oxidatively cross-linked, as in vancomycin, heterocyclized, as in penicillin or, more commonly, cyclized, as in fengycin. Cyclization seems to be the predominant way of constraining nonribosomally synthesized peptides. Because peptide cyclization from the point of view of chemical synthesis is difficult to achieve without protection of all of the side chains, there has been rapidly growing interest in exploration of the enzymatic cyclization mechanism for the development of new synthesis routes.

Naturally occurring macrolactones and macrolactams
The nonribosomal machinery for peptide synthesis uses large multienzyme complexes as an assembly line to catalyze stepwise peptide condensation. The substrates of these multienzyme complexes are not restricted to the 20 amino acids, since hundreds of building blocks are now known to be integrated and modified by postsynthesis action. Common to this assembly line is the incorporation of nonproteinogenic amino acids, such as D-isomers, carboxy acids, and N-methylated residues, as well as the incorporation of heterocyclic rings and fatty acids. Glycosylation and oxidative cross-linking are common further postsynthetic modifications by enzymes that are associated with the NRPS machinery.

In order to understand the principles of this enzymatically directed peptide synthesis, the mechanistic features of NRPS are briefly summarized with the surfactin synthetase from Bacillus subtilis. The surfactin synthetase is a large multienzyme complex consisting of three enzymatic subunits, SrfA (402 kDa), SrfB (401 kDa), and SrfC (144 kDa), which consist of seven modules that comprise 24 catalytic domains. Each module is responsible for the specific incorporation of one dedicated substrate into the growing heptapeptide chain. The N-terminal module of an assembly line, the initiation module, specifically recognizes and activates the N-terminal amino acid of the peptide product. All chemical reactions necessary to incorporate and modify each substrate are mediated by a catalytically independent set of domains incorporated within the modules. The first step in biosynthesis is the recognition and activation of a dedicated substrate by the adenylation domain (A domain; about 550 amino acids). By analogy to aminoacyl-tRNAsynthetase, the
A domain catalyzes the activation of a substrate as aminoacyladenylate through the Mg\(^{2+}\)-dependent hydrolysis of ATP and the release of pyrophosphate\(^{55}\). In the next step, the aminoacyladenylate intermediate is transferred to the free thio group of the cofactor phosphopantetheine, which is tethered to the thiolation domain (T domain, or peptidyl carrier protein; about 80 amino acids) located downstream of the A domain\(^{57-58}\). The phosphopantetheine arm is attached to an invariant serine residue of the apo-T domain by a dedicated 4’-phosphopantetheine (ppan) transferase that uses coenzyme A (CoA) as a substrate\(^{59-60}\). The intermediates, tethered by the reactive thioester to the flexible cofactor phosphopantetheine (in each module), can be transferred to other domains for subsequent catalytic reactions. Peptide bond formation between two adjacent substrates is catalyzed by the condensation domain (C domain; about 450 amino acids), which is located between the A and T domains of subsequent modules\(^{61}\). The C domain catalyzes the nucleophilic attack of the amino acid bound to the downstream T domain with its free \(\alpha\)-amino group on the activated thioester of the upstream T-domain-bound intermediate\(^{61}\). For the surfactin initiation reaction, peptide bond formation occurs between modules 1 and 2 by the nucleophilic attack of the \(\alpha\)-amino group of leucine on the thioester-activated carboxy group of glutamate to give a dipeptide which is then translocated to module 2.

All known NRPS macrocyclization strategies lead to cyclic or cyclic branched-chain peptides. In macrolactones, the branch point can be either a hydroxylated amino acid side chain or a hydroxylated fatty acid moiety. For the surfactin peptide cyclase, the ring closure is
enzymatically catalyzed between an N-terminal β-hydroxyl fatty acid and the C-terminal peptide end. Cyclization was observed only when the (R)-β-hydroxyl fatty acid was used, while the (S)-enantiomer showed only enzymatic hydrolysis, indicating stereoselective recognition. The lipodepsipeptide cyclic product is a strong detergent with antiviral, hemolytic, and antibacterial activities. A major contribution to the detergent activity is provided by the lipopeptide chain, which is believed to be transferred to the N-terminal residue during the initiation reaction by a fatty acid acyltransferase. In contrast, the syringomycin (Pseudomonas syringae) and fengycin (B. subtilis) lipopeptide cyclases accept serine and tyrosine side chains of the peptide sequence as nucleophiles for cyclization, discriminating the N-terminal β-hydroxyl group of the attached fatty acid moiety. Moreover, these peptide cyclases display a very high level of regioselectivity by selecting only one specific residue of the substrate from a large source of nucleophiles for cyclization.

In addition to macrolactonization, natural product diversity also is increased by various enzymatic macrolactamization strategies. Basic head-to-tail peptide macrolactamization is observed in the antibiotic tyrocidine from Bacillus brevis and the potent immunosuppressive drug cyclosporin A from Tolypocladium niveum. In cyclosporin A, the final peptide bond is formed by a putative condensation domain instead of a peptide cyclase, emphasizing that nature developed two enzyme species capable of catalyzing product release by cyclization. Besides head-to-tail cyclized lactams, branched-chain lactams also are observed. The peptide cyclase of the antifungal lipopeptide mycosubtilin from B. subtilis forms
regioselectively an amide bond between an N-terminal β-amino fatty acid and the peptide C-terminal end. As in surfactin, in mycosubtilin a fatty acid chain is involved in the cyclization process, providing an amine as a nucleophile. While the precursors in both cases seem to be β-keto fatty acid residues derived from fatty acid synthases, nature processes these ketones in different ways. They are either reduced to a hydroxyl group, as observed in surfactin, or they are reductively aminated in a process catalyzed by aminotransferases, as observed in mycosubtilin. In both cases, nature uses a common precursor motif, which is subsequently diversified by the application of different synthetic strategies to increase the product outcome. As in fengycin and syringomycin, enzymatic amide bond formation also can occur between an ornithine side chain and the C-terminal peptide end, as observed in the antibiotic bacitracin from Bacillus licheniformis. The scope of cyclic lactames and lactones can be further broadened by oligomerization of peptide monomers. This additional strategy enables the B. brevis gramicidin S peptide cyclase to cyclodimerize two linear pentapeptides by catalyzing two subsequent peptide bond formation steps to form the cyclic lactam antibiotic gramicidin S. Also, cyclotrimerization is observed for the siderophore-forming peptide cyclases of enterobactin and the bacillibactin synthetase from Escherichia coli and B. subtilis, respectively. Three units of 2,3-dihydroxybenzoyl-serine are fused together by three subsequent ester bond formation steps between the serine hydroxyl group of one molecule and the C-terminal end of another molecule to give the cyclic trilactone enterobactin. This lactone displays iron-chelating activity, which is closely related to its structure. Three intramolecular catechol ligands provide electron donors required for the
coordination of iron, once more emphasizing the close relationship between cyclic structural organization and biological activity.

NRPS peptide cyclases can generate diverse cyclic peptide molecules ranging in size from very small, as in pristinamycin, with 7 residues, to very large, as in syringopeptin, with 22 residues.\textsuperscript{75-76} At the large end of the scale is another source of macrocyclic molecules observed in nature, referred to as naturally occurring circular proteins\textsuperscript{77}. These proteins are of bacterial origin and have a folded three-dimensional structure. In contrast to NRPS, they are produced by the translation of genes. Cyclization occurs posttranslationally only in a head-to-tail fashion to produce a seamless circle of peptide bonds. In contrast to what is known for NRPS, not much is known about the cyclization mechanism of the linear precursors.

The cyclization strategies reported here emphasize that nature has developed a large enzymatic tool set which allows the introduction of diversity into linear peptide sequences by a variety of different cyclization steps. Selection from different nucleophiles, enantiomers, and positions in the peptide sequence makes peptide cyclases unique enzymatic tools with very specific intrinsic stereo- and regioselective recognition elements. Moreover, an understanding of the catalysis of one, two, or three subsequent condensation steps toward cyclization requires further studies of the structural and mechanistic aspects of these enzymes.
CHEMOENZYMATIC CYCLIZATION

The great pharmacological potential of many cyclic peptides emphasizes their role in drug discovery, as they show specific interactions with cellular targets and a high level of resistance to proteolytic enzymes. They are therefore most promising scaffolds for pharmacophores. So far, synthetic chemistry faces several difficulties in the production of cyclic compounds providing sufficiently good yields and regioselectivity. Although cyclization is an entropically favorable process, synthetic macrocyclization is difficult to achieve, since steric repulsion of ring residues as well as the use of protecting groups to ensure proper regiochemistry decreases yields and makes chemical synthetic operations expensive and rather difficult. Since nature developed stereo- and regioselective peptide cyclization enzymes, researchers have aimed to combine chemical linear peptide synthesis with enzymatically catalyzed cyclization. This approach allows easy synthesis of linear peptide sequences by established solid-phase peptide chemistry, followed by selective and efficient enzymatic cyclization without the use of protecting groups and the formation of undesirable by-products.

In order to achieve chemoenzymology, translation between the language of chemistry and the language of biology must be established by chemically mimicking the biological pathway as closely as possible. This was first achieved by Trauger et al., who cloned and overexpressed an excised peptide cyclase (28 kDa) from the tyrocidine synthetase\textsuperscript{78}. In order to prove the activity of this isolated enzyme versus that of the natural enzyme, which is embedded in a 724-kDa multienzyme complex, a short mimicked copy of the natural cofactor...
phosphopantetheine, N-acetylcysteamine (SNAC), was attached to the C-terminal end of a chemically synthesized linear tyrocidine peptide. SNAC represents a link between natural and artificial systems and is compatible with both. Incubation of the mimicked substrate and the excised peptide cyclase revealed activity with an observed cyclization/hydrolysis ratio of 6:1. The turnover of 59 min\(^{-1}\) indicated a very rapid conversion of the linear compound into the cyclic compound, indicating the usefulness of this enzyme as an in vitro biocatalyst\(^{79}\). Follow-up studies with various peptidyl-SNAC substrates having various lengths, stereochemical properties, and amino acid compositions revealed that the tyrocidine peptide cyclase recognizes only C- and N-terminal residues of the substrate on the basis of identity and stereochemistry, leaving space for making longer and shorter substrates as well as for replacements of residues within the peptide backbone\(^{80}\). A minimal recognition model was postulated\(^{81}\). This observed substrate tolerance of the excised tyrocidine peptide cyclase allowed the synthesis of diverse tyrocidine variants in which position 4 (D-Phe) was replaced by 1 of 96 natural and unnatural amino acids. This library of tyrocidine product analogs was subsequently screened for improved or altered bioactivity. In contrast to the natural antibiotic tyrocidine, which does not discriminate between bacterial and eukaryotic cell membranes, the screen revealed that the substitution of D-Phe at position 4 with a positively charged D-amino acid led to a 30-fold increase in the selective recognition of bacterial membranes\(^{82}\).

In addition to their use with the tyrocidine peptide cyclase, SNAC substrates also were used to characterize the cyclization of gramicidin S
and surfactin\textsuperscript{83-84}. In contrast to the tyrocidine peptide cyclase, the surfactin peptide cyclase showed much less substrate tolerance, indicating differences in the binding pockets of these enzymes. While the tyrocidine peptide cyclase was capable of cyclizing shorter and longer SNAC substrates, the surfactin peptide cyclase showed only SNAC hydrolysis for shorter and longer compounds. Moreover, a change in the N-terminal nucleophilic attacking group from an amine to a hydroxyl group in the tyrocidine sequence resulted in no change in the cyclization outcome, while an opposite change from a hydroxyl fatty acid to an amino fatty acid group in the surfactin sequence resulted in only hydrolysis and not cyclization. An important feature of cyclic peptides is the $\beta$-sheet content, which is high for molecules with $(4n + 2)$ residues\textsuperscript{85-86}. A peptide with a high $\beta$-sheet content, such as the dekapeptide tyrocidine ($n = 2$), facilitates cyclization through substrate preorganization by backbone-to-backbone hydrogen bonds. This intrinsic property of tyrocidine facilitates easy cyclization, which was also reported to occur without catalysis, but at a lower efficiency\textsuperscript{87}. In contrast, the heptapeptide surfactin displays fewer $\beta$ sheets and therefore no substantial substrate preorganization. The $\beta$-sheet contents in the peptide sequences of surfactin and tyrocidine therefore also may contribute to the observed differences in substrate tolerance.

To expand the set of cyclization catalysts, peptide cyclases from other NRPS systems, such as mycosubtilin and fengycin from \textit{B. subtilis} and syringomycin from \textit{P. syringae}, recently were cloned and overexpressed. Contrary to the observations for the surfactin and tyrocidine cyclases, no activity was observed for the mycosubtilin,
fengycin, and syringomycin cyclases with synthetically made peptidyl-SNAC substrates, indicating a limitation in the chemoenzymatic potential of the latter cyclases. The inability to recognize or bind SNAC substrates in the active site of the excised peptide cyclase could be affected by the manner in which the short mimicked copy of SNAC is presented to the enzyme. To overcome this limitation, the cyclase domain (TE domain) was excised with the preceding cofactor-binding T domain as a T-TE didomain. Recombinant apo-T-TE cyclases then could be loaded in vitro with chemically synthesized peptidyl-CoA by using the ppan transferase Sfp. The resulting peptidyl-ppan-T-TE holocyclase carried the covalent cofactor-bound substrate in a way that mimicked the natural substrate presentation in the NRPS assembly line (28). Incubation of fengycin T-TE cyclase with fengycin CoA and Sfp revealed cyclization and hydrolysis activities, which were not observed with SNAC substrates alone. These results indicate that the peptide needs to be directed into the peptide cyclase active site by the cofactor ppan to ensure correct enzyme recognition; in contrast, soluble substrates are not properly directed into the active site by diffusion. Cyclases which do not show activity with SNAC substrates seem to require covalent binding of the peptide substrate to the ppan T-domain in order to catalyze cyclic product formation.
STRUCTURAL AND MECHANISTIC ASPECTS OF NRPS
PEPTIDE CYCLASES

The three-dimensional organization of enzyme residues encodes all information required to understand the principles and general features of macrocyclization. Regiospecific selection of only one nucleophile for cyclization and the exclusion of water to prevent undesired hydrolysis are features which are embedded in the structural fold. Moreover, the question of how thioesterases from different NRPS systems catalyze termination in one case by cyclization and in another case by hydrolysis needs to be elucidated. Crystallographic data for the excised surfactin peptide cyclase (TE domain) showed that this enzyme is a member of the \( \alpha \),\( \beta \)-hydrolase family \(^9\). Since this was the first crystal structure determined for an NRPS peptide cyclase, it served as a prototype for detailed mechanistic investigations\(^9\). The structural similarity to serine hydrolases suggested that an active-site catalytic triad is responsible for the macrocyclization activity. This notion is in agreement with a recent structural model of the surfactin cyclase which suggested that the ppan T-domain-bound peptidyl chain is directed through a cleft into the active site of the peptide cyclase and transferred to an invariant serine residue (Ser80), which is activated by histidine (His207) and aspartate (Asp107) \(^9\). The identity of this catalytic triad was confirmed by mutational analysis, which showed that all three residues were essential for enzyme activity\(^9\). The hydrophobic surfactin peptidyl chain of the acyl-O-enzyme intermediate is accommodated in a predominantly hydrophobic binding pocket with two cationic residues predicted to direct cyclization through specific interactions with the substrate. Mutation of these residues
toalanine resulted in a dramatic decrease in overall activity, indicating their relevance for peptide recognition.

Further studies of enzymatic substrate recognition elements for the surfactin peptide cyclase were carried out by using a detailed substrate scan and cocrystallization analysis for enzyme-bound inhibitor-substrate. The crystallization studies revealed well-defined binding pockets for the two C-terminal leucine residues in the enzyme, while the rest of the peptide sequence seems to be less well coordinated. In the deacylation step of the reaction, the β-hydroxyl group of the fatty acid moiety is activated by the same histidine and aspartate to facilitate an intramolecular nucleophilic attack on the acyl-enzyme ester bond to release the final lactone product. Many thioesterase domains from other NRPS systems, e.g., vancomycin, as well as structurally related lipases only hydrolyze and do not cyclize their products. A sequence alignment between the surfactin peptide cyclase and other members of the αβ-hydrolase enzyme family that catalyze only hydrolysis revealed the conservation among lipases of a glycine residue involved in the formation of the oxyanion hole. In the surfactin peptide cyclase, a proline residue is located at this position. A mutation of proline to glycine resulted in a 12-fold change in the product ratio in favor of hydrolysis, indicating that the change from a rigid proline to a flexible glycine increases the conformational freedom in this region of the active site and creates more access for water to capture the acyl-enzyme intermediate. This residue seems to be a switch between hydrolysis and cyclization among αβ-hydrolases.
Detailed investigations of the surfactin peptide cyclase provided insights into mechanistic and architectural features of an enzyme which produces a branched cyclic lipodepsipeptide. Less is known about the mechanism and structure of other cyclases, particularly oligomerizing cyclases. A detailed mass spectrometric analysis was carried out for the last module of the enterobactin assembly line (EntF), containing a C-terminal peptide cyclase which catalyzes the cyclotrimerization of three 2,3-dihydroxybenzoylserine (DHB-Ser) units to give the cyclic trilactone enterobactin. In order to localize acyl-enzyme intermediates, an active-site histidine-to-alanine mutant enzyme with a very low substrate turnover was used. With this approach, it was possible to provide evidence for a covalent acyl-O-TE domain intermediate and demonstrate that the peptide cyclase is involved in two reactions: acyl-chain growth and cyclization. In the first steps of acyl-chain growth, DHB-Ser is transferred to the active-site serine of the peptide cyclase by the nucleophilic attack of active-site serine on the acyl-thioester of the upstream holo-T domain. The second step requires catalytic generation of a DHB-Ser alkoxide, which in turn allows nucleophilic attack on another DHB-Ser thioester-bound T domain to form a dimeric ester. The elongation step is repeated a third time before the final cyclic trilactone is released by the intramolecular nucleophilic attack of serine on the acyl-O-TE domain ester bond. This mechanistic analysis suggests that the enterobactin cyclase serves as a "waiting room" while the phosphopantetheinyl T domain is reacylated, a process which requires a stable ester bond and the exclusion of any water from the active site. Crystallographic data for this peptide cyclase and
others will provide more insight into the overall mechanisms and allow for a comparison of their features.

Research efforts of the past few years in the growing field of enzymatic peptide cyclization in nonribosomal peptide synthesis, summarized have revealed substantial insights into the architectural, mechanistic, and functional organization of NRPS peptide cyclases. Based on the diversity of natural cyclization strategies, chemoenzymatic approaches were developed to allow cross talk between biology and chemistry to reprogram natural peptide sequences by chemical peptide synthesis and subsequent enzymatic cyclization. This method can serve as a new source of small cyclic peptide molecules with altered or improved pharmacological activities. Since these enzyme catalysts are valuable tools for the synthesis of cyclic molecules, future research efforts also will concentrate on in vitro protein evolution to generate custom-made catalysts for cyclization of a given peptide sequence.

The uses of traditional medicinal plants for primary health care have steadily increased worldwide in recent years. Scientists are in search of new phytochemicals that could be developed as useful antimicrobials for treatment of infectious diseases. Currently, out of 80% of pharmaceuticals derived from plants, very few are now being used as anti-microbials. Plants are rich in a wide variety of secondary metabolites that have found anti-microbial properties. This review highlights the current status of traditional medicine, its contribution to modern medicine, recent trends in the evaluation of anti-microbials with a special emphasis upon some tribal medicine, in vitro and in
vivoexperimental design for screening, and therapeutic efficacy in safety and human clinical trails for commercial outlet. Many of these commercially available compounds are crude preparations administered without performing human clinical trials. Recent methods are useful to standardize the extraction for scientific investigation of new phytochemicals and anti-microbials of traditionally used plants. It is concluded that once the local ethnomedical preparations of traditional sources are scientifically evaluated before dispensing they should replace existing drugs commonly used for the therapeutic treatment of infection. This method should be put into practice for future investigations in the field of ethnopharmacology, phytochemistry, ethnobotany and other biological fields for drug discovery.

Primitive people have used plants to cure a variety of human ailments. Even today, 85% of Indians use higher plants as effective anti-microbials for the treatment of various diseases.96 A large number of anti-microbial agents derived from traditional medicinal plants are available for treating various diseases caused by micro-organisms.97 They are used to eliminate the infecting micro-organisms. The therapeutically useful novel agents should inhibit the germs and exhibit greater selective toxicity towards the infecting germ than the host cells.98 The mode of action for plant-derived agent should target biochemical features of the invading pathogens that are not possessed by the normal host cell. Some of the factors important for anti-microbial treatment include methods such as sensitivity of the infecting micro-organism to a particular agent.99 Side-effects of the plant-derived agent can be tested relative to direct toxicity upon animal cells.
because of their close association with human tissues or cells. So, we attempt to summarize information linked to plant extracts/chemical substances for the effective treatment of certain bacterial and fungal diseases. We also discuss the obvious necessity for new anti-microbial agents in various therapy regimens.

Anti-bacterial screening of traditional medicinal plants has been the source of innumerable therapeutic agents. In the area of antibiotics, random screening as a tool of discovering new biologically active molecules has been most productive. Chemotaxonomic considerations and target-directed screening also play a crucial role. For a successful outcome the main requirement is access to a large number of compounds/extracts that must be well screened. Ethanol extracts of 78 traditional medicinal plants from India are used for treating infectious diseases and show bacterial and fungal activity at 1.6 mg/ml. The 50% ethanol extracts of 285 plant materials were screened for 61 biological activities and revealed effective anti-bacterial, and a wide range of pharmacological, activities. Anti-microbial and phytochemical studies revealed 45 Indian medicinal plants effective against multi-drug-resistant bacteria. These results suggest the presence of either good anti-bacterial potency or the high concentration of an active principle in the extract. Plant extracts were screened phytochemically and 20% of the species yielded positive reactions for alkaloids, 25% species contained steroids/triterpenoids and 45% of species possessed saponins. Those plants with anti-bacterial effects are rich in polyphenolic substances such as tannins, catechins, alkaloids, steroids and polyphenolic acids. The anti-bacterial activity also could be due to various chemical components
and the presence of essential oils in adequate concentrations, which damage micro-organisms. The insolubility of essential oils and non-polar extracts make it very difficult for them to be used in an aqueous medium during the study of anti-microbial activity. A great number of factors can influence the results such as the extraction method, volume of media, culture composition and incubation temperature. However, the recent advanced method of bioautographic TLC assays makes it possible to localize anti-microbial activity on a chromatogram; while bioassay-guided fractions led to the isolation of compounds.

Traditional medicinal plants have an almost maximum ability to synthesize aromatic substances, most of which are phenols or their oxygen-substituted derivatives. Most of these are secondary metabolites, of which 12000 plant-derived agents have been isolated in the recent past. Many of these substances serve as plant defense mechanisms against invasion by micro-organisms (Table 1), insects and herbivores. Some of the plant substances such as terpenoids are responsible for odor (quinones and tannins) plus pigment of the plant. Many compounds are responsible for plant flavor (e.g. the terpenoid capsaicin from chili peppers), and some of the same herbs and spices used by humans to season food yield useful medicinal compounds. The useful major groups of anti-microbial phytochemicals can be divided into several categories that include alkaloids, flavones (flavonoids, flavonols, Quinones), essential oils, lectins, polypeptides, phenolics, polyphenols, tannins and terpenoids.
Heterocyclic nitrogen compounds are called alkaloids. The first medically useful alkaloid was morphine, isolated in 1805 from Paver somniferum (opium poppy). The name morphine comes from the Greek word Morpheus, which means ‘god of dreams’. Codeine and heroin are both derivatives of morphine. Diterpenoid alkaloids, isolated from the plants of the Ranunculaceae family, are commonly found to have antimicrobial properties. Bioassay-guided isolation studies done on the root extract of Polyalthia longifolia shows that it possesses significant antibacterial activity led to the isolation of three new alkaloids pendulamine A, pendulamine B and penduline along with stigmasterol 3-O-beta-D-glucoside, allantoin, the known diterpenoid kolavenic acid and the azafluorene alkaloid isoursuline. Compound pendulamine A and pendulamine B were found to be active. Micro-organism inhibition concentrations, abbreviated as MICs, are ~0.02–20 µg against bacteria. The seed pods of Erythrina latissima yielded erysotrine, erysodine, syringaresinol, vanillic acid and a new erythrina alkaloid, (+)-10,11-dioxoerysotrine that was lethal to brine shrimp. 2-(5'-Hydroxy-3'-methoxy phenyl)-6-hydroxy-5-methoxybenzofuran has strong anti-microbial activity against yeast spores. Ethanol extracts of the Guatteria multivenia root have furnished known alkaloids such as liriodenine, lyciscamine, lanuginosine, guadiscine and O-methylpallidine. Lanuginosine possesses weak inhibitory effects against fungi and liriodenine was found to have anti-microbial activity against both bacteria and Candida albicans. Pyrrolizidine alkaloids (Heliotropium subulatum) extracts showed anti-microbial activity against both fungal and bacterial species. Alkaloids isolated (Schizozygia coffaeoides) using bioassay-guided fractionation was isoschizogaline,
schizogynine and indoline that were subsequently shown to be the most active anti-fungal compounds. The anti-microbial berberine alkaloid isolated from Mahonia aquifolium was active against bacteria. For the anti-microbial components of berberine from Hydrastis canadensis, change in the lipophilicity of protoberberinium salts caused by modification of the substituents appears to influence the anti-bacterial activity. Both berberine and palmatine exhibited the greatest anti-bacterial activity. Biologically active carbazole alkaloids (from Murraya koenigii) showed mosquitocidal and anti-microbial activities, as well as exhibited topoisomerase I and II inhibition activities.

Flavones are phenolic structures containing one carbonyl group. They are hydroxylated phenolic substances that occur as C6–C3 units linked to an aromatic ring. Flavonoids are known to be synthesized by plants in response to microbial infection and are effective anti-microbial substances against a wide array of microorganisms. Anti-microbial flavonoids have been reported from E. latissima. Dimethoxyflavone and bonducellin were isolated from the aerial parts of Caesalpinia pulcherrima. Isobonducellin was found to be a homoisoflavanoid containing a cis (Z)-double bond possessing anti-microbial activity. Compounds of C. pulcherrima with anti-viral activities were derived from the flavonoid of quercetin. Moreover, the flavonoids, acacetin-7-o-β-D-galactopyranoside of C. morifolium was found to be active as towards HIV. A wide variety of flavonoids, sesquiterpenoid alcohols, triterpenoids and quinic acid caffeates product from plants may also be useful as anti-microbials. The activity is probably due to
their ability to form a complex with extra-cellular and soluble proteins, which then binds to bacterial cell wall. More lipophilic flavonoids may also disrupt microbial membranes. Flavonoids lacking hydroxyl groups on their β-rings are more active against microorganisms and the microbial target is the membrane with –OH groups.

The anti-microbial properties of aromatic volatile oils from medicinal, as well as other edible, plants have been recognized since antiquity. Essential oil, which is used as a food flavoring agent, possesses a broad spectrum of anti-microbial activities attributed to the high content of phenolic derivatives such as carvacrol and thymol. Some essential oils are used for systemic and superficial fungal infections and further exploration reveals a broad spectrum effect against other pathogenic manifestations that include malignancy. Moreover, fragrance of plants is associated with essential oils. This oil consists of secondary metabolites which are highly enriched in compounds based on an isoprene structure. They are called terpenes and occur as diterpenes, triterpenes, tetraterpenes as well as hemiterpenes and sesquiterpenes. When the compounds contain additional elements, usually oxygen, they are termed as terpenes. Terpenenes or terpenoids are active against bacteria. Nearly 60% of all essential oil derivatives possess inhibitory effects upon fungi while 39% inhibited bacteria. The seeds of Nigella sativa Linn. (Ranunculaceae) contain active constituents, e.g. volatile oil and thymoquinone showed protection against nephrotoxicity and hepatotoxicity induced by either disease or chemicals. The seed oil has anti-inflammatory, analgesic, anti-pyretic, anti-microbial and anti-neoplastic activity. Petroleum ether extract of Melicope indica afforded
two unusual pentacyclic triterpenes and the ubiquitous steroids, stigmasterol and sitosterol. Pentacyclic triterpenes were isolated from Combretum imberbe that are novel glycosidic derivatives (hydroxyimberbic acid). Terminalia stuhlmannii Engl. stem bark yielded two glycosides of hydroxyimberbic acid, several of which had antibacterial activity. Imberbic acid showed potent activity against Mycobacterium fortuitum and Staphylococcus aureus. New cycloartane-type triterpenes isolated from the aerial parts of Acalypha communis exhibited moderate anti-microbial activity (MIC 8 and 32 µg/ml) against vancomycin-resistant enterococci. Compounds tested in an in vivo model did not provide protection to mice infected with S. aureus. Friedelin, epifriedelinol, β-amyrin, β-sitosterol, β-sitosterol 3-β-D-glucopyranoside and naringin isolated from the methanol extract of dried rhizome from Drynaria quercifolia showed concentration-dependent broad spectrum of anti-bacterial activity. Andrographolide, neoandrographolide and andrographiside (Fig. 1) are the diterpene lactone of Andrographis paniculata (king of bitter) possesses liver protection under various experimental conditions. It showed weak antimicrobial activity against bacteria and viruses. Asiaticoide and hypaphorine (Fig. 1) are the mixture of pentacyclic triterpene of Centella asiatica. Topical and oral applications of asiaticoside improved wound healing in guinea pigs (1 mg/kg dose). Tinosporaside and columbin are diterpenes and cordifolioside is a sesquiterpene glucoside of Tinospora cordifolia as reported. An ether extract of the aerial part of T. cordifolia inhibited the growth of M. tuberculosis at 1 : 50 000 dilutions. The mechanism of action of terpenes
is not fully understood but it is speculated to involve membrane disruption by the lipophilic compounds.

The active compounds can be grouped into two major classes: anti-microbial proteins and a wide variety of non-protein compounds. Their distribution is often tissue specific and they are usually found in cells located at the external layers of plant tissues, thus suggesting that these compounds would be the first line of defense against a pathogen attack. An anti-microbial compound of 316 Da, present in the soluble fraction of strawberry (Fragaria ananassa) leaves shows in vitro activity against bacterial and fungal plant pathogens. An anti-microbial protein (WjAMP-1) purified from leaves of Wasabia japonica showed anti-microbial activity against both fungi and bacteria. Oleanolic acid (C. paraguariensis Burk, Fabaceae) from an Argentinean legume was found active against Bacillus subtilis and S. aureus with MICs 64 µg/ml. Peptides that are inhibitory to micro-organisms were first reported in 1942. Peptides called cathelicidins represent an important native component of innate host defense in mice and provide protection against necrotic skin infection caused by Streptococcus. They are often positively charged and contain disulfide bonds. The mechanism of action may be ion channel formation in the microbial membrane or competitive inhibition of adhesion of microbial proteins to host polysaccharide receptors. Diverse application has been demonstrated for anti-microbial peptides as anti-infective agents. The broad spectrum activity displayed by anti-microbial peptides is considered a ‘chemical condom’ against HIV infection and Herpes simplex virus.
Some of the simplest bioactive phytochemicals consist of a single substituted phenolic ring. Cinnamic and caffeic acids are common representatives of a wide group of phenylpropane-derived compounds that are in the highest oxidation state. The common traditional medicinal plants have such compounds that are effective against bacteria. Phenolic compounds possessing a C3 side chain at a lower level of oxidation and containing no oxygen are classified as an essential oil and reported as anti-microbials. Coumarins are phenolic substances made of fused benzene and α-pyrone rings. Several coumarins have anti-microbial properties and anti-viral effects reported in 1954. Anti-microbial properties of phenolic compounds (Finnish berries) active against pathogenic bacteria exhibited different sensitivities towards phenolics. These properties can be utilized in functional food development and for food preservation. Phenols are toxic to microorganisms because of the sites and numbers of hydroxyl groups on the phenol groups, which is all related to their relative toxicity of microorganism. There is evidence that highly oxidized phenols possess more inhibitory action. The mechanism responsible for phenolic toxicity to micro-organisms includes enzyme inhibition by the oxidized compounds, possibly through reaction with sulphydryl groups or through more non-specific interactions with proteins. Polyphenols which can form heavy soluble complexes with proteins may bind to bacterial adhesions thereby disturbing the availability of receptor on the cell surface.

Tannins are generally descriptive of a group of polymeric phenolic substances capable of tanning leather or precipitating gelatin from solutions, the property known as astringency. Their
molecular weights range from 500 to 3000 Da and are found in almost every plant parts: bark, leaf, root, wood and fruit. They form two groups, hydrolysable and condensed tannins based on gallic acid. The first group is usually found as multiple esters with D-glucose, while the more numerous condensed tannins are derived from flavonoid monomers. Tannins (tannic acid) are water-soluble polyphenols that are present in many plant foods. Polyphenols (Tea) and many tannin components have been suggested to be anti-carcinogenic. The anti-microbial activities of tannins are well documented. The growth of many fungi, yeasts, bacteria and viruses were inhibited by tannins. Tannic acid and propyl gallate inhibit food borne, aquatic and off-flavor-producing microorganisms. Their anti-microbial properties seemed to be associated with the hydrolysis of an ester linkage between gallic acid and polyols hydrolyzed after the ripening of many edible fruits. Tannins in these fruits thus serve as a natural defense mechanism against microbial infections. The anti-microbial property of tannic acid can also be used in food processing to increase the shelf-life of certain foods, such as catfish fillets. Tannin components of epicatechin and catechin (Vaccinium vitis-idaea L.) showed strong anti-microbial activity against bacteria and fungi. Such anti-microbial activity could potentially be used as a possible alternative for the treatment of periodontal diseases. Eucaglobulin is a new complex of gallotannin and monoterpane of leaves of Eucalyptus globulus possessing anti-bacterial effects. Methanol extracts of T. citrina fruit yielded known tannins such as corilagin, punicalagin and chebulagic acid that were tested for anti-microbial action. Arjunolic acid, ethyl gallate, flavone, ellagic acid and gallic acid are the active constituents of T. arjuna. Macro cyclic structures
of bioactive ellagiannins (gluconic acid core) and oligomeric ellagitannins have been found in species of Myrtaceae and Elaeagnaceae and they too possess anti-bacterial activity against Helicobacter pylori. Proanthocyanidins (condensed tannins) and hydrolyzable tannins are the two major classes of tannins. Proanthocyanidins are flavonoid polymers, the most common type of tannin found in forage legumes. Hydrolyzable tannins are polymers of gallic and ellagic acid esterified to a core molecule of Phyllanthus emblica. Phyllanthin and hypophyllanthin are lignans of P. niruri that enhance cytotoxic response against multi-drug-resistant cells. The mode of anti-microbial action of tannins and their ability to inactivate microbial adhesins, enzymes and cell envelop transport proteins also have been studied.

The neem (Azadirachta indica), verasingam pattai (Zanthoxylum limonella), Indian babool (Acacia nilotica) stick are widely used as tooth brushes by various tribes throughout India, Africa and Nigeria. The neem (A. indica), traditionally used as medicine, and in particular the stem bark extract showed activity against various Candida species. The minimum fungicidal concentrations ranged from 0.06 to >8 mg/ml. Anti-bacterial effects of neem mouthwash have been tested over a period of 2 months against salivary levels of Streptococcus mutans, Lactobacillus species and S. mutans. They were all inhibited by neem-based mouth washes. The active component of the Nigerian chewing stick (Fagara zanthoxyloides) was found to consist of various alkaloids. Ayurvedic practitioners rely on plant extracts, both single and mixed combination, for the preparation. The preparations are used to treat a wide range of human, as well as animal,
ailments. Cleistanthus collinus is known as oduvanthalai (Nillipalai) in India and all parts of this plant are highly poisonous. Various extracts of this plant yield a multitude of compounds that include glycosides, arylnathalene lignan lactones such as cleistanthin A and B, collinusin and oduvin found to have anti-microbial activities. Strawberry extracts were strong inhibitors of Salmonella bacteria. The dried flower-heads of Chrysanthemum moriforium are an oriental drug as well as a popular herbal tea in China, which has been used for the treatment of eye diseases in Japan. They have been found to possess anti-bacterial, anti-fungal, anti-viral and anti-inflammatory activities.

Traditional healers prepare a wide range of healing juices, crude extracts, paste and tincture from various herbs by using a water extract. Water or alcohol (methanol/ethanol) are mainly used for a large number of crude extract/library preparations (dry powder soaking or suspension, mechanical shaker, distillation of essential oils), sequential grinding (alkaloids, steroids, triterpenoids), gradial centrifugation (lectins and polypeptides) and acid hydrolysis (phenols) for a specific time frame. A variety of extractants are used for their ability to solubilize anti-microbials and also other factors from plants. This particular study provided a more standardized extraction method for a wide variety of plants. The crude extracts or mixtures of compound-rich residues are used for the initial screening of plants for anti-microbial activities. In many reports, methanol or ethanol are used for alkaloid extraction; acetone for flavonoids and steroids, hexane, diethyl ether and chloroform for fat soluble oils, wax, lipids and esters; dichloromethane for terpenoids, ethyl acetate for esters, ethanol for sterols, polyphenols,
tannins and water for the water soluble components like glycosides, polysaccharides, polypeptides and lectins, which are most effective against pathogens. TLC, other chromatography separations and several solvent systems are used for the elution of enormous water and organic solvent soluble anti-microbial compounds. Diverse analytical spectral devices are often used for the identification and structural characterization of active components from plants. However, the water medium is the most suitable for the treatment point of view in humans/animals.

Several plants used for the treatment of various infections have some in vitro activity against pathogenic Gram-positive bacteria. In our findings, the popular use of Tragia involucrata as a traditional medicine for the treatment of scabies and skin infection was documented in India. The most promising T. involucrata and its extracts (organic and water) exhibited marked and/or broad spectrum activity especially towards Gram-negative and -positive bacteria. These results suggested the presence of good anti-microbial potency and a high concentration of active principles that were proven useful for wound healing in a rat model. Anti-inflammatory properties and safety of extracts were also evaluated. Effective inhibition is due to the high content of active principles in the extract. Whereas, among catechins (a constituent of green tea) and pyrogallol (Holmskioldia sanguinea Retz, as well as other anti-fungal compounds like ginger (Zingiber officinale Roscoe) were found active against human pathogens. The use of natural remedies for the treatment of viral diseases has a long history in traditional systems of medicines. An extract of Ribes nigrum has been used as an ingredient in a
variety of food and folk medicines in Japan. The extract has activity which inhibits virus replication in cells, due to the inhibition of protein synthesis among infected cells from the very early stage of infection. Anti-protozoal and cytotoxic activities were also reported.

It is concluded that the traditional plants may represent new sources of anti-microbials with stable, biologically active components that can establish a scientific base for the use of plants in modern medicine. These local ethnomedical preparations and prescriptions of plant sources should be scientifically evaluated and then disseminated properly. The knowledge that primitive people used plants gives a clear idea about the unclear botanical preparation of traditional sources of medicinal plants. These can be extended for future investigation into the field of pharmacology, phytochemistry, ethnobotany and other biological actions for drug discovery.

In addition, folklorist, pharmacologist, phytochemists and ethnobotanists are investigating plants for anti-microbials. Several plant-based chemicals have shown potential inhibitory action on a wide range of micro-organism in vitro experiment. Although tannins, polyphenols, oils and others were identified as the effective component for the bacterial damaging or killing action in the in vitro system, many of these compounds failed in human clinical trails to determine their therapeutic effectiveness.

We concluded that well-designed bioassay-guided isolation and studying anti-microbial effect should be able to complete this task. In the past 20 years, several studies proving the extract or chemicals derived
from plant extracts were reported. These studies indicated that extracts contain interesting biopharmaceutical substances (anti-microbials) that have attracted significant scientific attention. More detailed investigation at molecular, cellular levels, suitable animal models and human clinical studies are necessary to elucidate anti-microbial and other biological activities.

Natural products are naturally derived metabolites and/or byproducts from microorganisms, plants or animals. These products have been exploited for human use for thousands of years and plants have been the chief source of compounds used for medicine. Even today the largest users of traditional medicines are the Chinese with over 5000 plants, and plant products in their pharmacopoeia. In fact, the world’s best known and most universally used medicinal is aspirin (salicylic acid) which has it natural origins from the glycoside salicin which is found in many species of the plant genera Salix and Populus. Examples abound of natural product use, especially in small native populations in a myriad of remote locations on earth. For instance, certain tribal groups in the Amazon basin, the highland peoples of Papua New Guinea, and the aborigines of Australia each has identified certain plants to provide relief of symptoms varying from head colds to massive wounds and intestinal ailments. History also shows that now extinct civilizations had also discovered the benefits of medicinal plants. In fact, nearly 3000 years ago, the Mayans used fungi grown on roasted green corn to treat intestinal ailments. More recently, the Benedictine monks (800 AD) began to apply Papever somniferum as an anesthetic and pain reliever as the Greeks had done for years before. Many people, in past times,
realized that leaf, root and stem concoctions had the potential to help them. These plant products, in general, enhanced the quality of life, reduced pain and suffering, and provided relief, even though an understanding of the chemical nature of bioactive compounds in these complex mixtures and how they functioned remained a mystery.

It was not until Pasteur discovered that fermentation is caused by living cells that people seriously began to investigate microbes as a source for bioactive natural products. Then, scientific serendipity and the power of observation provided the impetus to Fleming to usher in the antibiotic era via the discovery of penicillin from the fungus – Penicillium notatum. Since then, people have been engaged in the discovery and application of microbial metabolites with activity against both plant and human pathogens. Furthermore, the discovery of a plethora of microbes for applications that span a broad spectrum of utility in medicine (e.g. anticancer and immunosuppressant functions), agriculture and industry is now practical because of the development of novel, and sophisticated screening processes in both medicine and agriculture. These processes use individual organisms, cells, enzymes, and site directed techniques, many times in automated arrays, resulting in the rapid detection of promising leads for product development.

Even with untold centuries of human experience behind us and a movement into a modern era of chemistry and automation, it is still the case that natural product based compounds have had an immense impact on modern medicine since about 40% of prescription drugs are based on them. Furthermore, 49% of the new chemical products registered by the
FDA are natural products or derivatives thereof. Excluding biologics, between 1989 and 1995, 60% of approved drugs and pre-new drug application candidates were of natural origin. From 1983-1994, over 60% of all approved and pre-NDA stage cancer drugs were of natural origin as were 78% of all newly approved antibacterial agents. In fact, the world’s first billion dollar anticancer drug taxol is a natural product derived from the yew tree. Many other examples abound that illustrate the value and importance of natural products in modern civilizations.

Recently, however, natural product research efforts have lost popularity in many major drug companies and, in some cases, have been replaced entirely by combinatorial chemistry which is the automated synthesis of structurally related small molecules. In addition, many drug companies have developed interests in making products that have a larger potential profit base than antiinfectious drugs. These include compounds that provide social benefits, reduce the symptoms of allergies and arthritis, or ones that can soothe the stomach. It appears that this loss of interest can be attributed to the enormous effort and expense that is required to pick, and chose a biological source, then to isolate active natural products, decipher their structures, and begin the long road to product development. It is also apparent that combinatorial chemistry and other synthetic chemistries revolving around certain basic chemical structures is now serving as a never ending source of products to feed the screening robots of the drug industry. Within many large pharmaceutical companies, progress of professionals is primarily based upon numbers of compounds that can be produced and sent to the screening machines. This tends to work against the numerous steps needed even to find one
compound in natural product discovery. It seems important to realize that the primary purpose of combinatorial chemistry should be to complement and assist the efforts of natural product drug discovery and development, not to supersede it. The natural product often serves as a lead molecule whose activity can be enhanced by manipulation through combinatorial and synthetic chemistry. Natural products have been the traditional pathfinder compounds with an untold diversity of chemical structures unparalleled by even the largest combinatorial databases.

It may also be true that a reduction in interest in natural products for use in drug development has happened as a result of people growing weary of dealing with the traditional sources of bioactive compounds including plants of the temperate zones, and microbes from a plethora of soil samples gathered indifferent parts of the world by armies of collectors. In other words, why do something different (working on endophytic microbes) when robots, combinatorial chemistry and molecular biology have arrived on the scene? Furthermore, the logic and rationale for time and effort spent on drug discovery using a target-site directed approach has been overwhelming.

While combinatorial synthesis produces compounds at random, secondary metabolites, defined as low molecular weight compounds not required for growth in pure culture, are produced as an adaptation for specific functions in nature. Shutz notes that certain microbial metabolites seem to be characteristic of certain biotopes, both on an environmental as well as organismal level. Accordingly, it appears that the search for novel secondary metabolites should center on organisms
that inhabit unique biotopes. Thus, it behooves the investigator to
carefully study and select the biological source before proceeding, rather
than to have a totally random approach in the biological source
material. Careful study also indicates that organisms and their biotopes
that are subjected to constant metabolic and environmental interactions
should produce even more secondary metabolites. Endophytes are
microbes that inhabit such biotopes, namely higher plants, which is why
they are currently considered as a wellspring of novel secondary
metabolites offering the potential for medical, agricultural and/or
industrial exploitation. Currently, endophytes are viewed as an
outstanding source of bioactive natural products because there are so
many of them occupying literally millions of unique biological niches
(higher plants) growing in so many unusual environments. Thus, it would
appear that these biotypical factors can be important in plant selection
since they may govern the novelty and biological activity of the products
associated with endophytic microbes.

Since the discovery of endophytes in Darnel in 1904, various
investigators have defined endophytes in different ways which is usually
dependent on the perspective from which the endophytes were being
isolated and subsequently examined. Bacon and White give an inclusive
and widely accepted definition of endophytes—“Microbes that colonize
living, internal tissues of plants without causing any immediate, overt negative
effects”. While the symptomless nature of endophyte occupation in plant
tissue has prompted focus on symbiotic or mutualistic relationships
between endophytes and their hosts, the observed biodiversity of
endophytes suggests they can also be aggressive saprophytes or
opportunistic pathogens. Both fungi and bacteria are the most common microbes existing as endophytes. It would seem that other microbial forms most certainly exist in plants as endophytes, but no evidence for them has yet been presented e.g. mycoplasmas, and archebacteria. The most frequently isolated endophytes are the fungi. It turns out that the vast majority of plants have not been studied for their endophytes. Thus, enormous opportunities exist for the recovery of novel fungal forms, taxa, and biotypes. Hawksworth and Rossman estimated there may be as many as 1 million different fungal species, yet only about 100,000 have been described. As more evidence accumulates, estimates keep rising as to the actual number of fungal species. For instance, Dreyfuss and Chappela estimate there may be at least 1 million species of endophytic fungi alone. It seems obvious that endophytes are a rich and reliable source of genetic diversity and novel, undescribed species. Finally, in our experience, novel microbes usually have associated with them, novel natural products. This fact alone helps eliminate the problems of dereplication in compound discovery.

It is important to understand the methods and rationale used to provide the best opportunities to isolate novel endophytic microorganisms as well as ones making novel bioactive products. Thus, since the number of plant species in the world is so great, creative and imaginative strategies must be used to quickly narrow the search for endophytes displaying bioactivity.
A specific rationale for the collection of each plant for endophyte isolation and natural product discovery is used. Several reasonable hypotheses govern this plant selection strategy and these are as follows:

1. Plants from unique environmental settings, especially those with an unusual biology, and possessing novel strategies for survival are seriously considered for study.

2. Plants that have an ethnobotanical history (use by indigenous peoples) that are related to the specific uses or applications of interest are selected for study. These plants are chosen either by direct contact with local peoples or via local literature. Ultimately, it may be learned that the healing powers of the botanical source, in fact, may have nothing to do with the natural products of the plant, but of the endophyte (inhabiting the plant).

3. Plants that are endemic, having an unusual longevity, or that have occupied a certain ancient land mass, such as Gondwanaland, are also more likely to lodge endophytes with active natural products than other plants.

4. Plants growing in areas of great biodiversity also have the prospect of hosting endophytes with great biodiversity.
### TABLE 5.1

**ACTIVITY ORDER OF MACROCYCLIC COMPLEXES AND DIHYDRAZIDES**

<table>
<thead>
<tr>
<th>Against Bacteria</th>
<th>Against Fungi</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>1. Compounds of Thiodiacetic acid</strong></td>
<td></td>
</tr>
<tr>
<td>V (II) [DCCTDAH] (BF$_4$)$_2$</td>
<td>V (II) [DCCTDAH] (BF$_4$)$_2$</td>
</tr>
<tr>
<td>V (II) [DPTDADH] (BF$_4$)$_2$</td>
<td>V (II) [DPTDADH] (BF$_4$)$_2$</td>
</tr>
<tr>
<td>&gt;&gt; TDADH</td>
<td>&gt;&gt; TDADH</td>
</tr>
<tr>
<td><strong>2. Compounds of Thiodipropianic acid</strong></td>
<td></td>
</tr>
<tr>
<td>V (II) [DPTDPDH] (BF$_4$)$_2$</td>
<td>V (II) [DCCTDPDH] (BF$_4$)$_2$</td>
</tr>
<tr>
<td>V (II) [DCCTDPDH] (BF$_4$)$_2$</td>
<td>V (II) [DPTDPDH] (BF$_4$)$_2$</td>
</tr>
<tr>
<td>&gt;&gt; TDPDH</td>
<td>&gt;&gt; TDPDH</td>
</tr>
<tr>
<td><strong>3. Compounds of Iminodiacetic acid</strong></td>
<td></td>
</tr>
<tr>
<td>V (II) [DCCIDAH] (BF$_4$)$_2$</td>
<td>V (II) [DCCIDAH] (BF$_4$)$_2$</td>
</tr>
<tr>
<td>V (II) [DPIDADH] (BF$_4$)$_2$</td>
<td>V (II) [DPIDADH] (BF$_4$)$_2$</td>
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<tr>
<td>&gt;&gt; IDADH</td>
<td>&gt;&gt; IDADH</td>
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<tr>
<td><strong>4. Compounds of Oxydiacetic acid</strong></td>
<td></td>
</tr>
<tr>
<td>V (II) [DPODADH] (BF$_4$)$_2$</td>
<td>V (II) [DCCODAH] (BF$_4$)$_2$</td>
</tr>
<tr>
<td>V (II) [DCCODAH] (BF$_4$)$_2$</td>
<td>V (II) [DPODADH] (BF$_4$)$_2$</td>
</tr>
<tr>
<td>&gt;&gt; ODAH</td>
<td>&gt;&gt; ODAH</td>
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REFERENCES


(1999)

15. Gutierrez-Lugo MT, Singh MP, Maiese WM, Timmermann BN. 
New antimicrobial cycloartane triterpenes fromAcalypha 


17. Handa SS. Indian Herbal Phamacopoeia I:. Mumbai, India: 
Indian Drug Manufacturer's Association 102-B. 158–9. (1998)

Techniques of Plant Analysis London: Chapman and Hall. 1– 
302. (1998)

19. Heitz, M.C. Guillaumount, D. Bruand-Cote, I. Daniel C. 

20. Hirasawa M, Takada K. Multiple effects of green tea catechin 
on the antifungal activity of antimycotics againstCandida 

Phys., in press.


43. Kunle O, Okogun J, Egamana E, Emajevwe E, Shok M. Antimicrobial activity of various extracts and carvacrol


49. Mahady GB, Pendland SL, Yun GS, Lu ZZ, Stoia A. Ginger (Zingiber officinale Roscoe) and the gingerols inhibit the


82. Scholz-Schroeder, B. K., Soule, J. D. Lu, S. E. Grgurina, I. and Gross. D. C. A physical map of the syringomycin and syringopeptin gene clusters localized to an approximately 145-


94. Trauger, J. W., Kohli, R. M. and Walsh. C. T. Cyclization of backbone-substituted peptides catalyzed by the thioesterase


