CHAPTER 1

Introduction to Pharmaceutical Chemistry of Natural Products

INTRODUCTION

For thousands of years, natural products (drugs and pharmaceutical substances obtained from natural origin) have played vital role in treating and preventing human diseases. Humans always have been interested in naturally occurring compounds from prebiotic, microbial, plant and animal sources. In earlier period of treatment, naturally obtained ingredients were believed to be combined with witchcraft or mysticism. Later these treatments were found effective and the results were documented in the development of early herbal medicine. The science of pharmacognosy (a branch of pharmaceutical science), which deals with the naturally obtained drugs, developed from these records to provide an authentic, scientific description of the natural products used in treating and preventing human ailments.

The term *Pharmacognosy* was coined by a German scientist from two Greek words *pharmakon* (means a drug) and *gignosco* (means to acquire the knowledge of), in the year 1815. Pharmacognosy is defined as the 'study of the physical, chemical, biochemical and biological properties of medicinal products obtained from our living environment as well as the search for new drugs from natural sources'.

Pharmacognosy is an established basic pharmaceutical science, which has changed considerably from being largely descriptive botanical and mycological field to having more of chemical and biological focus. It serves as an important linkage between basic biology and medicinal chemistry. The advent of phytochemistry and pharmacological screening programme (heart of the drug discovery process from crude extracts) helps the natural products to find their way into medicine as purified phytochemicals, rather than in the form of conventional preparations.

Chemistry of Natural Products is a branch of chemistry that deals with the isolation, identification, structure elucidation and study of the chemical characteristics of chemical substances produced by living matters. The study of pharmaceutical applications of these natural products led to the establishment of a new avenue called *Pharmaceutical Chemistry of Natural Products*.

DRUG DISCOVERY FROM NATURAL PRODUCTS

Investigation of biological components of plant products and other organisms are carried out by adopting any of the five recognized approaches as given:

1. Random screening.
2. Selection of specific taxonomic groups, such as families or genera.
3. Chemotaxonomic approach, in which some classes of secondary metabolites, such as terpenoids, alkaloids, proteins, etc., are selected.
4. Information-managed approach, in which some of the species are selected based on database surveillance.
5. Selection by an ethnomedical approach (based on its use in traditional medicine).

The value of natural products in the field of medicine can be assessed using the following three criteria:

1. The rate of introduction of New Chemical Entities (NCEs) of wide structural diversity, including serving as templates for semi synthetic and total synthetic modification using these natural products.
2. The number of diseases treated or prevented by these substances.
3. The frequency of use of these natural products in the treatment of diseases.

Table 1.1 Drugs derived from natural sources

Natural products	*Medicinal uses*
Acarbose	Antidiabetic
Artemisinin	Antimalarial
Azithromycin	Macrolide antibiotic
Cefetamet pivoxil	Antibacterial
Cefozopran	Antibacterial
Cefpimizole	Antibacterial
Clarithromycin	Macrolide antibiotic
Irinotecan	Anticancer
Ivermectin	Antiparasitic
Miglitol	Antidiabetic
Mizoribine	Immunosuppressive
Mycophenolate mofetil	Immunosuppressive
Paclitaxel	Anticancer
Pentostatin	Anticancer
Policosanol	Nutritional supplement
Sirolimus	Immunosuppressive
Tacrolimus	Immunosuppressive
Teicoplanin	Antibacterial
Vinorelbine	Antimitotic
Topotecan	Topoisomerase I inhibitor
Voglibose	Alpha-glucosidase inhibitor

An analysis of the origin of the drugs developed between 1981 and 2010 showed that natural products or natural product-derived drugs comprised 36% of all NCEs launched into the market. In addition, 28% of the NCEs were synthetic or natural mimic compounds, based on the study of pharmacophores (the section of the molecule containing the essential organic functional groups which directly interact with the receptor-active site and hence, confers the desired biological activity) related to natural products. Between 1990 and 2000, a total of 41 drugs derived from natural products were launched in the market by major pharmaceutical companies (Table 1.1); some of these drugs are azithromycin, paclitaxel, sirolimus (rapamycin), synercid, tacrolimus and topotecan.

The above analysis suggests that the natural products are an important source for new drugs and are also good lead compounds suitable for further modification during drug development. The large proportion of natural products in drug discovery has stemmed from the diverse structures and then intricate carbon skeletons of natural products. Since secondary metabolites from natural sources have been elaborated within living systems, they are often perceived as showing more 'drug-likeness and biological friendliness than totally synthetic molecules', making them good candidates for further drug development.

Nomenclature

The chemical structures of natural products are generally complex in nature, some are remarkably complex and hence naming the compound by systematic nomenclature will not be possible. Therefore, the names are given on the basis of trivial nomenclature, in which the discoverer has the right to name the compound. In general, the source of the compound is selected to supply the root name, e.g. digoxin and digitoxin from *Digitalis purpurea*. The suffix *-in* indicates 'a constituent of', *-oside* to show the compound is a sugar derivative (e.g. glycoside, sennoside), *-genin* for the aglycone obtained upon hydrolysis of the sugar derivative (e.g. digoxigenin), *-toxin* for a poisonous constituent (e.g. podophyllotoxin), or may reflect chemical functionality, such as *-one* (e.g. menthone) or *-ol* (e.g. menthol). Conventionally *-ine* is always used for alkaloids or amines (e.g. quinine and reserpine). The analogues are then named as derivatives of the original natural compound by using the standard prefixes like *hydroxy-*, *methoxy-*, *methyl-*, *dihydro-*, *homo-*, etc., for added substituents to the main compounds and *deoxy-*, *demethyl-*, *demethoxy-*, *dehydro-*, *nor-*, etc., for removed substituents from the main compounds. Some groups of compounds like steroids, fatty acids and prostaglandins are named by adopting the systematic and conventional approach from an accepted root name. For example, the name *cholesterol* (cholest-5-en-3α-ol) is given on the basis of stem name *cholestane*.

Classification of Natural Products

The pharmacologically useful bioactive compounds of natural origin can be classified into various categories in a different manner. The classification of natural products is described as follows:

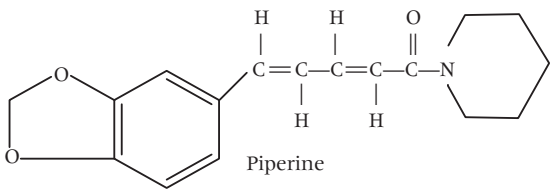
Chemical classification

Based on the chemical nature of the secondary metabolites, they may be classified as alkaloids, terpenoids, flavonoids, etc. Further, each of these can be classified on the basis of its chemical nature, like open-chain aliphatic, alicyclic and cycloparaffinic, aromatic, benzenoid and heterocyclic.

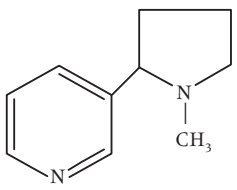
For example, the classification of alkaloids is depicted as given:

Heterocyclic alkaloids

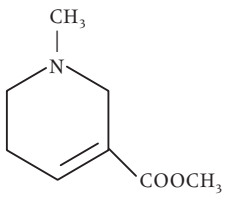
1. Pyridines and piperidines



Piperine

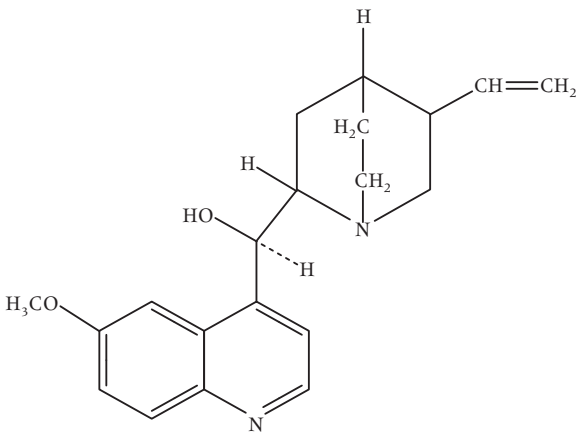


Nicotine

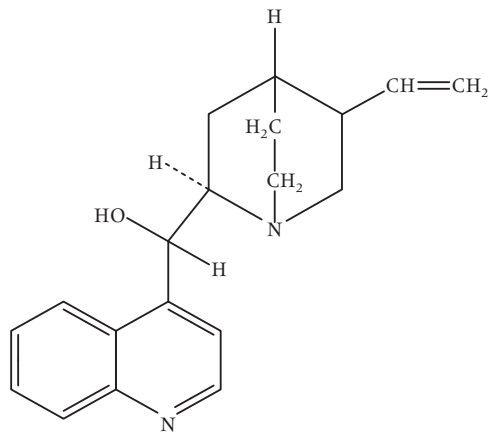


Arecoline

2. Quinolines

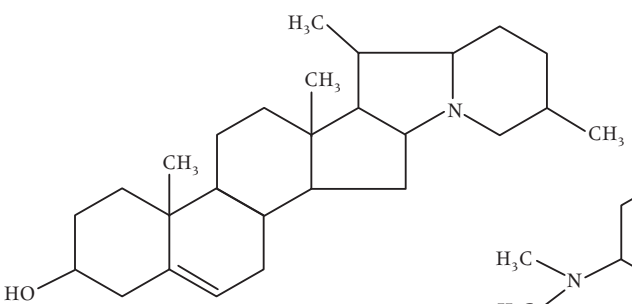


Quinine

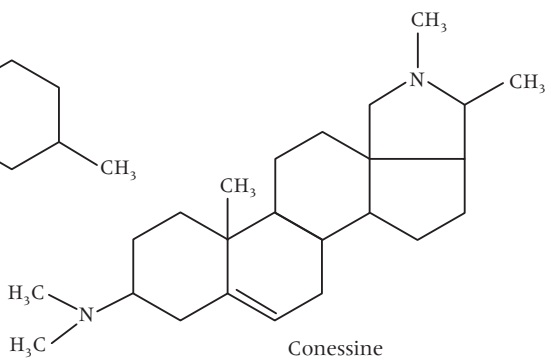


Cinchonine

3. Steroidal alkaloids

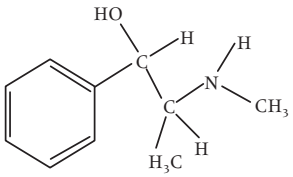


Solanidine

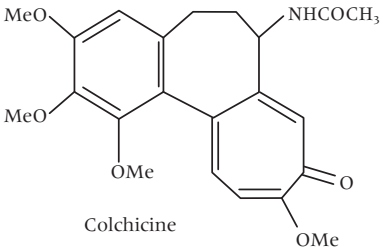


Conessine

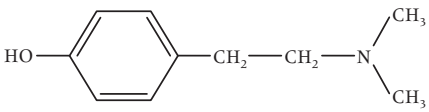
Nonheterocyclic alkaloids



Ephedrine



Colchicine



Hordenine

Pharmacological classification

Natural products are frequently initiated by attempts to isolate and clarify a pharmacologically active principle of plant or animal origin. They may be classified on the basis of its therapeutic activity as given:

Analeptics	:	Strychnine, Brucine
Analgesics	:	Morphine
Anticancer	:	Vincristine, Taxol
Antimalarial	:	Quinine
Local anaesthetic	:	Cocaine

Chemotaxonomic classification

This is performed on the basis of the plants and the chemical nature of the natural products. Chemotaxonomy serves as markers for the evolution as well as the classification of plants. The characters generally studied in chemotaxonomy are secondary metabolites of pharmaceutically important compounds like alkaloids, glycosides, flavonoids, etc.

Biogenetic classification

The primary synthetic process in nature is photosynthesis by which green plants utilize the energy of the sun for the production of organic compounds from carbon dioxide. The initial products of photosynthesis are carbohydrates. Further metabolic alterations lead to the formation of a pool of organic compounds of low molecular weight and simple structures such as carboxylic (-COOH) and amino acid ($NH_2CHRCOOH$) groups, which are vital for all the living organisms. It is responsible for synthetic starting materials for specific, genetically controlled, enzymatically catalysed reactions that lead to the complex compounds that characterize the secondary metabolism of plants and mammals. The reaction pathway leading to a particular natural product is called the *biosynthetic pathway* and the corresponding event is known as *biogenesis*. Different plant and animal species can employ different biosynthetic pathways to produce the same metabolite. This feature can be employed in the classification of plants in terms of their chemotaxonomy.

Among the four major classes of biochemicals (carbohydrates, proteins, nucleic acids and lipids), experiments have indicated that the first three classes could have arisen through prebiotic chemistry. Although the biosynthesis of many natural products can be traced back to acetate (e.g. fatty acids, terpenes and polyketide biosynthesis) or amino acids (e.g. alkaloid biosynthesis), there are many whose biosynthetic origins are either obscure or result from a complex combination of multiple synthetic pathways.

Identification of Natural Products

Medicinal chemistry has evolved from the chemistry of bioactive compounds in early days due to the works at the interface of chemistry and biology. Medicinal chemistry of bioactive natural products spans a wide range of fields, including isolation and characterization of bioactive compounds from natural sources, structural modification for optimization of the exerted activity and other physical properties and semisynthesis and synthesis for a thorough scrutiny of structure–activity relationship (SAR). In addition, synthesis of natural products also provides a powerful means in solving supply problems in clinical trials and marketing of the drug. Obtaining natural products in bulk amount is often very difficult.

In general, chemistry of natural product work is initiated after a given crude drug formulation (typically prepared by solvent extraction of the natural material) is judged 'active' in a particular in vitro assay. If the end goal of the work at hand is to identify which one(s) of the hundreds of compounds are responsible for the observed in vitro activity, the path to that end is fairly straightforward. Steps involved are as follows:

1. Fractionate the crude extract, e.g. by solvent partitioning or chromatography.
2. Test the fractions thereby generated with in vitro assay.
3. Repeat steps (1) and (2) until pure, active compounds are obtained.
4. Determine structure(s) of active compound(s), typically by using spectroscopic methods.

Sometimes in vitro activity does not necessarily translate to activity in humans or other living systems. In that case, a typical protocol to isolate a pure chemical agent from natural origin is bioassay-guided fractionation, meaning step-by-step separation of extracted components based on differences in their physicochemical properties and assessing the biological activity, followed by the next round of separation and assaying.

The unique properties of natural products are disobedience to Lipinski's 'Rule of Five', which has been widely recognized as the most useful 'drug-like' compounds selection criteria. Drug discovery community began to once more appreciate the value of natural products and revived natural products research by integrating rapid isolation and identification with hyphenated technologies, parallel synthesis, computations and many other new techniques into medicinal chemistry of natural products.

Techniques Involved in Natural Product Lead Discovery

The typical process of discovering natural-product drugs and their progression towards development is depicted in Figure 1.1. In this generic scheme, the natural product is extracted from the source, concentrated, fractionated and purified, yielding essentially a single biologically active compound.

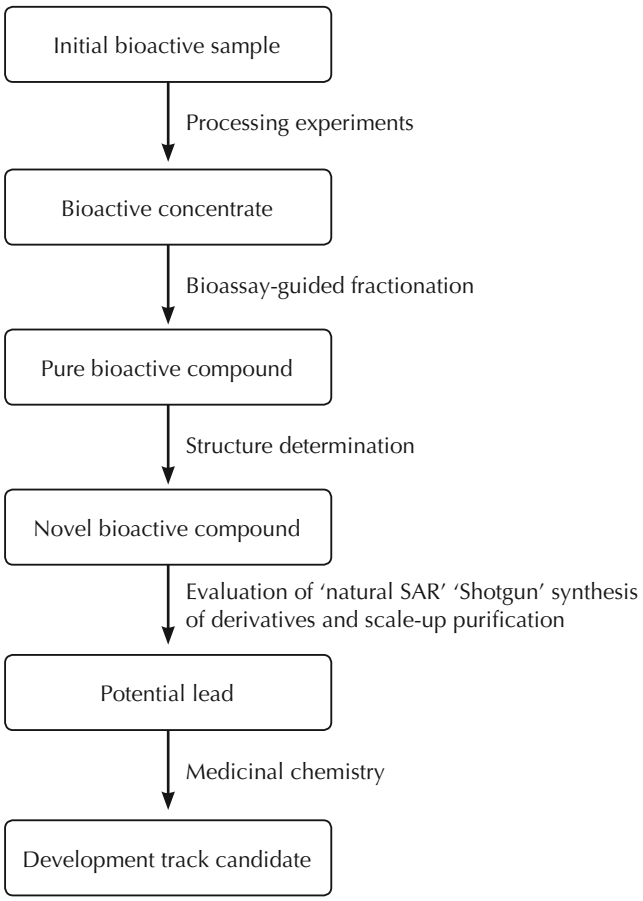


Figure 1.1 Chemical process for natural product drug discovery

Historically, this process has most often suffered from three major hurdles. The first is the rapid identification of known compounds (dereplication) to avoid duplication of effort. This step has been greatly facilitated by the availability of reliable directly coupled HPLC-mass spectrometer (LC-MS) systems, and the general availability of natural product databases. The pivotal development responsible for the success of LC-MS has been the introduction of efficient and general methods for producing ions from the effluent of HPLC separations. The most general of these methods, known as *electrospray ionization* (ESI) and *atmospheric pressure ionization* (API), can generate the ions essential for mass spectrometric analysis for greater than 90% of analytes, ranging from amino acids to proteins and nucleic acids. The correlation of both molecular mass and UV absorption data with known compounds by database searching is ordinarily sufficient to classify sets of compounds.

The second major hurdle in the process—the de novo structure determination of compounds that are (NMEs)—is an area that has been revolutionized by many advances in spectroscopic techniques, particularly in high-resolution NMR technologies. Of the many NMR advances, those

of particular importance to natural product structure determination fall into one of the given two main areas: multidimensional pulse methods and sensitivity improvements. From its inception, and particularly since the advent of two-dimensional NMR methods, high-resolution NMR spectroscopy has seen continuous development and expansion of the array of experimental methods available to elucidate chemical structures. New experiments, particularly multidimensional ones, provide scalar (through bond) ^{1}H-^{1}H and ^{1}H-[^{13}C, ^{15}N, ^{31}P] correlations and ^{1}H-^{1}H dipolar (through space) molecular connectivity data that essentially map out the structure of the compound. In the area of sensitivity, stronger magnetic fields provided by superconducting magnets, cryogenic electronics and micro-probe technologies have dramatically lowered the amount of material needed for structural analysis, to less than a milligram.

The combination of cryogenic probe electronics with correlation spectroscopy enables the development of further more powerful experiments, such as correlation experiments for low-abundance ^{13}C and ^{15}N nuclei, which are unattainable with conventional hardware. Determination of molecular formula is crucial to the process and is typically done by high-resolution mass spectrometry on microgram quantities of material. One of the most powerful of these techniques is Fourier transform ion cyclotron resonance mass spectrometer (FT-ICR MS), which is capable of measuring molecular mass with exceptional accuracy. Combining the tools of high-resolution mass spectrometry with two-dimensional NMR spectroscopy allows structure determination to be carried out on sub-milligram or milligram amounts of a compound in a matter of hours or days, rather than weeks or months. Although the determination of complex structures is technically challenging, it is no longer a major impasse in the drug discovery process. In the cases in which the biological activity profile meets criteria for potency and selectivity, preliminary SAR studies are conducted and the process is scaled up. A second avenue for exploring SAR in an expeditious manner is the 'shotgun' approach to chemical derivatization. The knowledge gained through understanding the natural products' SAR and the shotgun approach provided an early foundation on which the overall synthetic strategy was developed. Once the feasibility of modulating biological response through synthetic modification is established, the hit is declared a lead and proceeds for additional optimization by traditional medicinal chemistry.

Natural Products as Pharmacological Tools

There are many historical examples in which the natural product has not just been the medicinal product but has also helped revealing a novel aspect of physiology. For example, digitalis from foxglove showed the role of *sodium-potassium-ATPase*; morphine pointed the way to the receptors affected by endogenous opioids; muscarine, nicotine and tubocurarine helped to explore the different types of acetylcholine receptors, etc. More recently, there has been interest in systematically searching for small-molecule inhibitors of key steps in biochemical processes (chemical genetics). Given that many assays involve identifying phenotypic changes in living cells (as opposed to binding interactions with isolated proteins), it is probable that natural products will provide useful probes for such studies. Moving beyond observations of phenotypic changes to defining the alterations in gene expression or protein function that are responsible will require advances in transcriptomic and proteomic methods.

In summary, research on natural products has contributed significantly and has been the most successful strategy for discovering new drugs and for extending human life and improving clinical practice. As the chemical techniques improved dramatically, the active constituents are isolated from natural resources, characterized and synthesized in the pharmaceutical chemistry laboratory. These chemical modifications (semisynthesis) lead to find more active or better tolerated drugs. Gradually, the synthetic compounds superseded many of the natural products, but certain natural products were never surpassed and remain as valued medicines till today. Natural products play an important role in drug discovery programme, as long as nature continues to yield novel, diverse chemical entities possessing selective biological activities.