CHAPTER-I

Introduction to natural products and bioactive compounds:
A review
1.1. Introduction to natural products and bioactive compounds

Organic chemistry is an art executed science. The art of synthesis and isolation of natural products and bioactive compounds makes it one of the most interesting and finest areas of modern chemistry.

As an eminent scientist says, *If we wish to catch with nature, we shall need to use the same methods as she does, and I can foresee a time in which physiological chemistry will not only make greater use of natural enzymes but will actually resort to creating synthetic ones*.—Emily Fischer, 1902. These words reflected an extensive chemical synthesis and isolation of natural products and bioactive compounds for the welfare of human kind in the future thereafter.

The key role played by plant-based systems in the healthcare of different cultures has been extensively documented, and the World Health Organization (WHO) has estimated that approximately 65-80% of the world’s population rely mainly on plant-derived traditional medicines for their primary health care.¹ Most of these natural products are secondary plant metabolites. Keeping this in view, we felt an urge to synthesize some natural products and test their bioactivity and also planned for the isolation of some bioactive natural compounds.

Natural products have played a key role in health care and prevention of diseases for the past thousands of years. Several ancient civilizations, such as Indians, Chinese, and North Africans have provided written evidence for the use of natural sources for curing
various ailments. Sumerian clay tablet is known to be the earliest known written document that was used as a remedy for various illnesses.

The importance of natural products as anticancer agents can be seen between the years 1981-2006, where about a hundred anticancer agents have been developed, of which, nine were pure natural products, eleven were derived from a natural product pharmacophore, eighteen were natural product mimics, and twenty five were natural product derivatives, thus making the natural sources as significant contributors to the health care system.

A few words below convey a subtle idea about the importance of natural products as medicines over the last 4000 years.

2000 BC: Here, eat this root.
1000 AD: That root is heathen! Here, say this prayer.
1850 AD: That prayer is superstition! Here, drink this potion.
1935 AD: That potion is snake oil! Here, swallow this pill.
1975 AD: That pill is ineffective! Here, take this antibiotic.
2000 AD: That antibiotic is poison! Here, eat this root.

-Aonymous

1.2. Characterization of natural products and bioactive compounds

The characterization of natural products and bioactive compounds in the past decades has utilized spectroscopic techniques as well as chemical methods to determine the structures. The use of ultraviolet-visible and infrared spectrophotometry, nuclear magnetic resonance
spectroscopy (NMR), mass spectrometry (MS), high performance liquid chromatography (HPLC), polarimetry, and circular dichroism can yield complementary information that is used to determine the structures of the compounds. When NMR data and degradative studies are inconclusive, a total synthesis and spectroscopic comparison between the synthetic and natural product is the easiest way for stereochemical determination and confirmation.\(^5\)

### 1.3. Dereplication

Dereplication is the rapid identification of known compounds within a sample. These known compounds interfere with *in vitro* assays, leading to false-positive results in bioassays. In order to avoid known or interfering compounds, various dereplication methods are applied to the bioactive samples. The currently used popular dereplication methods include mass spectrometry (MS)-based methods, NMR-based methods, chromatographic methods, and activity profiling.

#### 1.3.1. **MS-based methods:**

It includes liquid chromatography-MS (LC-MS), LC-UV-MS, and LC-MS-NMR methods. These methods allow for an easy determination of the molecular weights of compounds in either a purified or crude sample.\(^6\) In some methods, the LC-MS, UV, and bioactivity profiles of a particular crude extract can be compared with a library of characterized compounds, and a particular known active compound can be rapidly identified.\(^7,8\)
1.3.2. NMR based methods:

These rely on the ability of NMR-based techniques to resolve signals given by different functional groups within a molecule, or different molecules in a mixture, and help in the rapid identification of functional groups and structural motifs. DEPT, COSY or HSQC spectra are some of them.\textsuperscript{9,10}

1.3.3. Chromatographic methods:

Prefractionation methods depend on the ability to selectively remove a few undesirable classes of compounds. Selective removal of tannins by use of polyamide chromatography is one of the most widely used prefractionation-dereplication methods. Sephadex LH-20 and HP20 MCI gel\textsuperscript{11} were been reported to selectively remove tannins from plant extracts. The remaining extract can then be evaluated for bioactivity.

1.3.4. Activity profiling:

It is a dereplication method where the bioactivity of an extract is profiled in several different assays. The COMPARE algorithm used with the NCI's sixty-cell line panel is one of the most well-known methods. COMPARE method considers IC\textsubscript{50} values, which represent the concentration of a compound inhibiting the cell growth by 50%.\textsuperscript{12}

1.4. Biogenesis and classification of natural products:

The classification of natural products can be made on the basis of biogenesis where the process of photosynthesis plays a lead role. Carbohydrates, the initial products of photosynthesis undergo alterations and leads to the formation of low molecular weight pool of
organic compounds. The final natural product is formed from a sequence of biosynthetic reactions and the process is termed as biogenesis (Fig. 1.1).\textsuperscript{13}

![Biochemical pathway diagram](image)

**Fig. 1.1**

**1.5. Sources:**

Natural products generally have a prebiotic origin or they originate from plants, microbes, or animal sources (Fig. 1.2).\textsuperscript{14,15} Plants and microorganisms such as fungi, bacteria have proven to be an excellent source of novel natural products including peptide antibiotics,
polyketides and classes of other bioactive compounds. Some of the microbial metabolites are used as antineoplastic agents, antimicrobial agents and bioinsecticides.

![Diagram of natural product sources](image)

**Fig. 1.2**

The marine environment is a rich source of natural bioactive compounds as more than 70% of earth’s surface is covered by oceans. Thus we broadly have four categories of natural product sources.

**1.5.1. Natural products from plant sources:**

Since ages, plants have been an excellent source for the bioactive natural products. Several plant extracts were used as medicines in the treatment of various diseases. Higher plants are extremely popular for the production of variety of biologically active compounds that include, paclitaxel 1, morphine 2 and others.
1.5.2. Natural products from marine organisms:

Spongouridine (3) and spongothymidine (4)\textsuperscript{23} were known to be the first bioactive nucleotide compounds to be isolated from the Carribean sponge \textit{Cryptotheca crypta} in 1950’s. These compounds were potent anticancer and antiviral agents. Some of the marine organisms lead a sedentary lifestyle, and thereby synthesize several complex and extremely potent chemicals for their defense from predators.\textsuperscript{20} The potential of these chemicals can be utilized to treat various ailments, especially cancer. Discodermolide (5), isolated from the marine sponge \textit{Discodermia dissoluta}, is one such example which has a similar mode of action when compared to that of paclitaxel and possesses a strong anticancer activity. Infact, a combination therapy of the above two drugs has led to reduced tumor growth in certain cancers.\textsuperscript{24}

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{chemical_structures.png}
\caption{Chemical structures of Spongouridine (3), Spongothymidine (4), and Discodermolide (5).}
\end{figure}

1.5.3. Natural products from microorganisms:

The discovery of penicillin in 1929 opened the gates for microorganisms as a source of potential drug candidates. Since then, a large number of microorganisms were screened for drug discovery which led to the discovery of antibacterial agents like cephalosporins-
Cefprozil (6), antidiabetic agents like acarbose (7), and anticancer agents like epirubicin (8).\textsuperscript{25}

\textbf{1.5.4. Natural products from animal sources:}

Animals also form a source of some interesting bioactive compounds. Epibatidine (9), a nitrogen containing heterocyclic compound isolated from the skin of an Ecuadorian poison frog, was found to be ten times more potent than morphine.\textsuperscript{26} Venoms and toxins from animals have played a significant role in designing a multitude of cures for several diseases. Teprotide, a toxic venom, extracted from a Brazilian viper, has led to the development of cilazapril (10) and captopril (11), which were found to be effective against hypertension.\textsuperscript{27}
1.6. Bioassay:

Once a compound is synthesized or isolated, it needs to be screened for its biological activity. Screening is an attractive and key area towards the drug discovery programmes.\textsuperscript{28} It is crucial for successful isolation of bioactive compounds. Generally, bioassays are broadly classified into two types. They are mechanism based assays and cell based assays.\textsuperscript{29}

1.6.1. Mechanism based assays:

It involves the testing of a specific drug against a specific enzyme, DNA, receptor etc. As these assays are conducted in artificial environment which is different from physiological environment, it must be properly configured for accuracy. A properly handled assay can determine the activity of a compound even at lower concentrations.\textsuperscript{30} Though these assays are useful in determining the biological activity of the compounds, the disadvantage is that they approximate the \textit{in vivo} environment due to incomplete system generated from the absence of certain pathways.

1.6.2. Cell based assays:

It involves a drug-cell interaction with the complete intact cell rather than just isolated system and is by far more superior to the mechanism based assay. This method has the advantage of screening
of compounds that cannot pass through the cell membrane.

Though several classifications of bioassays are seen, one of the easier schemes\textsuperscript{31} to represent a screening is depicted below (Fig. 1.3).

Screening or bioassay is a powerful tool to analyze the activity and thus can give an idea of a compound to become a pharmacophore. It is done in combination with separation techniques (chromatography) and structural elucidation methods (spectroscopy). It is said that pharmacology at a lower dose is toxicology and toxicology at a higher dose is simply pharmacology. One of the bioassays is the test against
brine shrimp model. *In vivo* lethality towards an organism can be used as a convenient method for screening of bioactive compounds.\textsuperscript{32}

### 1.7. Bioactive natural products:

Natural products play a major role in organic chemistry. Some of these may possess biopotency, selectivity and pharmacokinetic traits making them drug agents. In some cases their analogues are accessed through modification that serves the requirements.

**Fig. 1.4**

The small molecule natural products are evolutionarily optimized to interact with either enzymes or receptors and other biomacromolecules making a pharmaceutical connection.\textsuperscript{33} As these
SMNP are valuable sources, these can either be isolated in smaller quantities from the natural source, or can be synthesized to meet the required needs (Fig 1.4). For the purpose of preparing these compounds the notion of diverted total synthesis approach was developed (Fig. 1.5). 34-36

![Syntheses diagram](image)

**Fig. 1.5**

Polyketides are one of the good examples for small molecule natural products. These are secondary metabolites from bacteria, fungi, plants and animals that are biosynthesized through the decarboxylative condensation of acetyl coenzyme A, as well as the compounds derived from them by further condensations. Some of these polyketides have attractive biological activities and are used as pharmacophores.

Picromycin (12), the first isolated macrolide showed interesting antibiotic properties. 37-39 Sporostatin (13) and xestodecalactones A–C (14-16) are ring keto lactones that bear a fused 1,3-dihydroxybenzene ring. Sporastatin or M5032 was isolated from *Sporormiella* sp. and was shown to be an inhibitor of cyclic adenosine 3,5-monophosphate phosphor-diesterase (cAMP-PDE) and epidermal growth factor (EGF) receptor tyrosine kinase. 40,41 Xestodecalactones A–C were first isolated...
from the *Penicillium* fungus which in turn is found in the marine sponge *Xestospongia exigua*. Xestadecalactone B (15) was shown to be active against *Candida albicans*.

Apart from these polyketides several other compounds isolated from various sources have shown potent biological activities. For instance, a cyclic octapeptide, curcacycline-A (17) isolated from *Jatropha* species showed immuno-suppressive activity and another compound curcacycline-B (18) from the same source enhanced rotamase activity of cyclophilin B. Chevalierin-A (19), a cyclic peptide isolated from *Jatropha chevalieri* was found to possess antimalarial activity.

Quinine (20), isolated from the Cinchona bark is one of the earliest natural compounds in the fight against malaria which also served as a template for the development of analogues such as chloroquine (21),
 primaquine (22), mepacrine (23) and mefloquine (24).\textsuperscript{46,47} These analogues were proved to be efficient antimalarials (Fig 1.6).

\begin{center}
\begin{tabular}{c c c c}
 & Chloroquine (21) & Primaquine (22) & Mepacrine (23) \\
Cl & & & \\
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\begin{center}
\begin{tabular}{c c c c}
 & Quinine (20) & Mefloquine (24) \\
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\textbf{Fig. 1.6}

Artemisinin (25),\textsuperscript{48-50} isolated from \textit{Artemesia annua}, is an antimalarial used successfully against chloroquine resistant malarial parasites. Its poor solubility led to the development of analogues artemether (26)\textsuperscript{51} and artesunate (27)\textsuperscript{52} that were oil and water soluble respectively.

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\textbf{Artemisinin (25) \hspace{1cm} Artemether (26) \hspace{1cm} Sodium artesunate (27)}

Apart from the several natural products that show varied biological activities, there are other bioactive compounds that are synthetically prepared either resembling few natural products or keeping in view of the active core fragment of the natural products. Substituted
pyrrolidines, pyrimidines are one of them. The substituted spiro pyrrolidines (28-32 and 33-38) (Fig. 1.7) that were prepared synthetically has shown a good antibacterial activity against various pathogens.53

![Substituted piperidines](image)

**Fig. 1.7**

Substituted piperidines are amongst the most ubiquitous heterocyclic building blocks in both natural products and synthetic compounds with important biological activities.55,56 Considering the extensive range of biological activities these compounds exhibit, it is not surprising that between 1988 and 1998 thousands of piperidine-derived compounds were mentioned in clinical and preclinical studies.57

Simple 2,6-disubstituted piperidines [solenopsins (39) and isosolenopsins (40)], isolated from fire ant venom, are reported to possess a broad range of activities such as antibacterial, necrotic, insecticidal, antifungal and anti-HIV.58 4-Hydroxy-2,6-disubstituted piperidine alkaloid, dendrobate alkaloid-241D (41), isolated from methanolic skin extracts of poison frog *Dendrobates speciosus* and *Dendrobates pumilio* possess potent biological activity.59,60 Synthetic racemic alkaloid-241D and the parent 4-piperidone (42) were found to
be potent inhibitors for binding of perhydrohistrionicotoxin to nicotinic receptor channels of electroplax membranes. In addition, racemic alkaloid-241D has been proved to be a non-competitive blocker of acetylcholine to ganglionic nicotinic receptor channels.

1.8. Natural products as anticancer drugs:

Natural products serve as the mother for several anticancer agents available in the market today. Podophyllotoxin (43), isolated from *Podophyllum peltatum* in 1944 was one of the early known compounds that worked as anticancer agent. Initially it served therapeutically as a purgative and in the treatment of venereal warts. Later studies proved that it acts as an anticancer agent by binding irreversibly to tubulin. Etoposide (44) and teniposide (45), were the modified analogs of podophyllotoxin, that cause cell death by inhibition of topoisomerase II, thus preventing the cleavage of the enzyme- DNA complex and arresting the cell growth. These analogs are used in the treatment of various cancers.
Catharanthus roseus, a member of the Apocynaceae family, is a rich source of indole alkaloids which include the anticancer vincristine (46) and vinblastine (47), and also the antihypertensive alkaloid, ajmalicine (48). Both vinblastine and vincristine are known to prevent cell division by inhibiting mitosis in the cell cycle and bind irreversibly to tubulin, thereby blocking cell multiplication and eventually causing cell death.\(^\text{68}\)

Camptothecin (49), isolated from Camptotheca acuminata, is an anticancer agent which has a unique mode of action. The low water solubility nature of camptothecin was overcome by preparing its water-soluble analogs, namely, topotecan (50) and irinotecan (51). All
of these compounds are topoisomerase-I inhibitors, and cause cell death by DNA damage.\textsuperscript{69}

\begin{center}
\begin{tikzpicture}
\node[align=center,anchor=west] (mychem) {\textbf{Camptothecin (49)} \hspace{1cm} \textbf{Topotecan (50)} \hspace{1cm} \textbf{Irinotecan (51)}};
\end{tikzpicture}
\end{center}

An extract of the Pacific yew tree, \textit{Taxus brevifolia} was found to possess excellent anticancer properties. The active component, paclitaxel (1), was isolated from the extract by Monroe Wall and Mansukh Wani.\textsuperscript{70,71} Paclitaxel promotes microtubule stabilization by binding irreversibly to $\beta$-tubulin.\textsuperscript{72} Cell multiplication requires equilibrium between tubulin-microtubule and its stabilization causes programmed cell death.\textsuperscript{73} Paclitaxel was the first compound to promote microtubule formation. It has been used in the treatment of ovarian and breast cancers as well as non-small cell lung tumors. The epothilones (52, 53), isolated from \textit{Sorangium cellulosum}, possess potential anticancer properties and also activity against taxane-resistant cell lines.

\begin{center}
\begin{tikzpicture}
\node[align=center,anchor=west] (mychem) {\textbf{Epothilone A (52)} \hspace{1cm} \textbf{Epothilone D (53)}};
\end{tikzpicture}
\end{center}
1.9. Natural products as antimicrobial drugs:

Canthin-6-one (54) isolated from *Allium neapolitanum* and *Zanthoxylum chiloperone* displayed a broad spectrum of activities against *Aspergillus fumigatus*, *A. niger*, *A. terreus*, *Candida albicans*, *C. tropicalis*, *Cryptococcus neoformans*, *Geotrichum candidum*, *Saccharomyces cerevisiae*, *Trichosporon beigeli*, *Trichosporon cutaneum* and *Trichophyton mentagrophytes var. interdigitale* with MIC values between 1.66 and 10.12 mg mL\(^{-1}\) whereas 5-Methoxycanthin-6-one (55) displayed selective activity against only *T. mentagrophytes var. interdigitale*, with an MIC value of 3.075 mg mL\(^{-1}\).\(^ {74}\)

YM-215343 (56), a cytotoxic compound found in the bacterial culture of *Phoma* sp. exhibited antifungal activity against some pathogenic fungi *C. albicans*, *C. neoformans* and *A. fumigatus*, with MIC values of 2–16 mg mL\(^{-1}\).\(^ {75}\) Zheng *et al.* isolated five epidithiodioxopiperazines, bionectins A (57), B (58) and C (59) and verticillins D (60) and G (61), from the cultures of the fungus *Bionectra byssicola* F120. Out of these compounds, 57, 58 and 60 exhibited antibacterial activity against *S. aureus* including methicillin resistant *S. aureus* (MRSA) and quinolone-resistant *S. aureus* (QRSA), with MIC values of 10–30 mgmL\(^{-1}\), while 59 showed no antibacterial activity even at 100 mg mL\(^{-1}\).\(^ {76}\)
Ratjadone 62, a polyketide isolated from cultures of Sorangium cellulosum strain Soce360, displayed potent in vitro antifungal activity with MIC values in the range from 0.004 to 0.6 µg/mL for Mucor hiemalis, Phytophthora drechsleri, Ceratocystis ulmi, and Monilia brunnea.
1.10. Natural Products as anti-HIV agents:

(E)-3-(3-Hydroxymethyl-2-butenyl)-7-(3-methyl-2-butenyl)-1H indole (63), a diprenylated indole alkaloid, isolated from the twigs and leaves of *Glycosmis montana* exhibited potent anti-HIV activity with an IC50 value of 1.17 mg/mL and an SI of 11.68. Anibamine (64), a novel pyridine quaternary alkaloid, was isolated from the stems of an *Aniba* species as a TFA salt, competed for the binding of 125I-gp120 to human CCR5 with an IC50 of 1 mM.

A few carbazole alkaloids, *O*-methylmukonal (65), 3-formyl-2,7 dimethoxycarbazole (66) and clauszoline J (67), isolated from the rhizomes and roots of *Clausena excavata* showed anti-HIV-1 activity in a syncytial assay, with EC50 values of 12.0, 29.1 and 34.2 mM and TI of 56.7, 8.0 and 1.6, respectively. Gomisin J (68), (-)-gomisin M1 (69), (+)-gomisin M2 (70) and schisanhenol (71) were isolated from the fruits of *Schisandra rubriflora*. These were found to be
dibenzocyclooctadiene lignans. Out of these lignans, (-)-gomisin M1 (69) exhibited the most potent anti-HIV activity, with EC50 and TI values of <0.65 mM and >68, respectively. It was found from the studies that compounds with aromatic hydroxyl groups were more active than those lacking aromatic hydroxyls, suggesting the importance of the aromatic hydroxyl groups for the anti-HIV activity of dibenzocyclooctadiene lignans. The position of the hydroxyl group was also a key for enhancing the anti-HIV activity, as 69 (with a 3′-OH), was more potent than 70 (with a 3-OH). 81

Thus the study of natural products and bioactive compounds becomes an interesting area of organic chemistry.
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