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Science of Natural Product in Drug Discovery- A Brief Review

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Abstract

Natural products and their related moieties have historically been incredible as a source of therapeutic agents. In last 5-10 years, research into natural products in the pharmaceutical industry has reduced, owing to issues such as the lack of compatibility of traditional natural-product extract libraries with high-throughput screening. It has long been recognized that natural-product structures have the characteristics of high chemical diversity, biochemical specificity and other molecular properties that make them favourable as lead structures for drug discovery, and which serve to differentiate them from libraries of synthetic and compounds. Recent advances in genomics and structural biology during the past decades are painting a clearer picture of the diversity of proteins targeted by natural-product molecules. Besides these, current lead generation strategies have led to a renewed interest in natural products in drug discovery.Drug discovery leading to robust and viable lead candidates' remains a challenging scientific task, which is the transition from a screening hit to a drug candidate, requires expertise and experience. Natural products and their derivatives have been recognized for many years as a source of therapeutic agents and ofstructural diversity. However, in addition to their chemical structure diversity and their biodiversity, the development of new technologies has revolutionized the screening of natural products in discovering new drugs.

Applying these technologies compensates for the inherent limitations of natural products and offers a unique opportunity to re-establish natural products as a major source for drug discovery. The present article attempts to describe the utilization of compounds derived from natural resources as drug candidates, with a focus on the success of these resources in the process of finding and discovering new and effective drug compounds, an approach commonly referred to as "natural product drug discovery"

Key Words: Natural products; Compatibility of natural extract; drug discovery; traditional natural product' therapeutic agents

Introduction

Natural products, including plants, animals and minerals have been the basis treatment of human diseases .Nevertheless, ancient wisdom has been the basis of modern medicine and will remain as one important source of future medicine and therapeutics. History of medicine dates back practically to the existence of human civilization. Historically, the majority of new drugs have been generated from natural products (secondary metabolites) and from compounds derived from natural products. Before 20th century, crude and semi-pure extracts of plants, animals, microbes and minerals represented the only medications available to treat human and domestic animal illnesses. The 20th century revolutionized the thinking in the use of drugs, as the receptor theory of drug action. The idea that effect of drug in human body are mediated by specific interactions of the drug molecule with biological macromolecules (proteins or nucleic acids in most cases) led scientist to the conclusion that individual chemical compounds in extracts, rather than some mystical "power of life" are the factors required for the biological activity of the drug. This lead to the beginning of a totally new era in pharmacology, as pure, isolated chemicals, instead of extracts, became the standard treatments for diseases. Indeed, many bioactive compounds, responsible for the effects of crude extract drugs, and their chemical structure was elucidated. Classical examples of drug compounds discovered this way are morphine, the active agent in Opium, and digoxin, a heart stimulant originating from flower Digitalis lanata. The evolution in synthetic chemistry also led to chemical synthesis of many of the elucidated structures. On the other hand, the R&D thrust in the pharmaceutical sector is focused on development of new drugs, innovative/indigenous processes for known drugs and development of plant-based drugs through investigation of leads from the traditional systems of medicine .

The exploitation of structural chemical databases consisting of a wide variety of chemotypes, in conjunction with databases on target genes and proteins, will surely facilitate the creation of new chemical entities through computational molecular modeling for pharmacological evaluation.Recently, there has been a renewed interest in natural product research due to the failure of alternative drug discovery methods to deliver many lead compounds in key therapeutic areas such as immunosuppression, antinfectives, and metabolic diseases. Natural products research continues to explore a variety of lead structures, which may be used as templates for the development of new drugs by the pharmaceutical industry. There is no doubt that natural products have been, and will be, important sources of new pharmaceutical compounds. Although traditionally natural products have played an important role in drug discovery.

Historical overview

Throughout the ages, humans relied on natural products. Natural products have earliest records from 2900-2600 BC documenting the uses of approximately 1000 plants derived substances such as the oil of Cedrus species (cedar), Commiphora myrrha (myrrh), Cupressus sempervirens (cypress), Glycyrrhiza and Papaver somniferum (poppy) .In addition to plants around 120 minerals were listed including Arsenic sulphide, Sulphur, Lime, Potassium permanganate and even rock salt. The first Egyptian record is 'Ebers papyrus' dating from 1500 BC, document about 850 drugs such as Aloe vera (aloe), Boswellia carteri (frankincense) and oil of Ricinus communis (castor). At the same time the Chinese 'Materia medica' was documented dating

from 1100 BC This is an Open Access article which permits unrestricted noncommercial use, provided the original work is Bing Fang with 52 prescriptions. Further the Greeks and Romans with Hippocrates (father of medicine) ~ 460 to 377 BC cover use of natural products which includes Extract of poppy, Henbane, Mandrake, Juniper and Saffron. Dioscorids (100 AD) compiled De Materia medica, which described the dosage and efficacy of about 600 plants derived medicines and laid the foundation of pharmacology in Europe. In 5 to 12 century the Arabs published their work in 'Canon medicinae' influenced by work of Ibn-Al-Baiter . Natural products chemistry actually began with the work of Serturner who first isolated Morphine from opium poppy (Papaver somniferum) in 1803 . Subsequent conversion into heroin was reported by Wright in 1874 . In 1817 Emetine was isolated from Ipecacuanha .Further other alkaloids such as Strychnine (Strychnos nux vomica) , Quinine(Cinchonaofficinalis) , Colchinne (Colchicum autumnale)], Atropine (Atropa belladonna)] ,Papaverine (Papaver somniferum) etc were isolated. No historical perspective of natural products derived drugs would be complete without discussion of Aspirin (acetyl salicylic acid). Mac lagan in 1876 introduced the salicin from extract of Salix or Spiraea ulmaria. Bergmann reported first antiviral agent Spongouridine and Spongothymidine from spong. The first antibiotic derived from natural products is the serendipitous discovery of Penicillin from Penicillium notatum (fungus) by Alexander Fleming in 1928.

Nature as source of new Drug compound

Despite the rise of combinatorial chemistry as an integral part of lead discovery process, natural products still play a major role as starting material for drug discovery. Drug product have been obtained from various sources which include plants, animal, marine and microbial metabolites.

1. PLANT SOURCES

Plants have been the part of traditional medicine systems, which have been used for thousands of years in our county.These plant based systems continue to play an essential role in health care, and it has been estimated by the World Organization (WHO) that approximately 80 % of the world's inhabitants rely mainly on traditional medicines for their primary health care. Plant products also play an important role in the health care systems of the remaining 20 % of the population, mainly residing in developed countries and at least 119 chemical substances, derived from 90 plant species, can be considered as important drugs currently in use in one or more countries. Of these 119 drugs, 74 % were discovered as a result of chemical studies directed at the isolation of the active substances from plants used in traditional medicine. Some examples are:

(a) ANTI-INFLAMATORY AGENTS

Inflammation is known to be one of the important causes responsible for many diseases. Natural products used for inflammation includes Withanolides from Withania somnifera. They are found to be active in arthritis and are potent inhibitor of angiogenesis, inflammation and oxidative stress. Inhibition of NFkB and NfkB regulated gene expression is primarily responsible for their anti arthritis example is Salai guggal (Boswellia serrata) which was investigated at IIM, JAMMU and also show anti arthritis action. Alkaloid,

berberine from Berberis aristata also have anti inflammatory action by inhibition of NFkB , COX2, TNFα , IL-Iβ, IL- 6. Another prominent example is Curcumin from Turmeric Curcuma longa , reported in 1971 to be an effective anti-inflammatory agent at CDRI LUCKNOW, show broad spectrum activity on inflammation. Another substance is Guggulsterone from Commiphora mukul (guggul). Nimbidin from neem (Azadirachta indica) and Embel a constituent of Vidang (Embelia ribes) also show anti-inflammatory action .

(b) CARDIO-VASCULAR AGENTS

Cardiac glycosides or cardenolides are commonly used. They are steroidal in nature with a lactone group. They inhibit the membrane bond Na-K ATPase pump resulting in depletion of intracellular K and increase in serum K which result in decrease electrical conductivity through a decrease in heart rate and increase cardiacoutput. Yellow oleander plant (Thevetia neriifolia) have thevitin A, B and peruvoside which are potent cardiac glycoside . Rauwolfia serpentine contain reserpine, was first tested in INDIA for anti-hypertensive activity. It inhibits action by inhibiting mono amine oxidase(MAO). The Terminalia arjuna bark has been used for treatment of angina. Arjunolic acid is main constituent to exhibit this action. The Coleus spp have also been reported in materia medica for treatment of heart disease. Digoxin obtained from Digitalis purpurea is most widely used cardenolides . Another most important example is Quinidine from Cinchona officinalis use as antiarrythmic agent

(c) ANTI DIABETIC AGENTS

India is a 'Diabetic capital of world' several remedies are used for their treatment. Most common example is Charantin a steroidal saponin have an insulin like activity . Sylvestre Gymnema (gurmar) from which gymnemic acid is obtain known to show hypoglycaemic activity .Futher Momordica charantia commonly known as Karela have momordicoside which is used for diabetes .

Andrographolide a di terpenoid lactone from graphis Paniculata has been found to exhibit significant hypoglycaemic activity . Syzygium jambolanium have anthocyanins which are responsible for antidiabetic action . Liquiritigenin extracted from Pterocarpus marsupium also another important example . Trigonella foneum–graecum commonly known as fenugreek, shows potent anti diabetic action .

(d) ANTI OBESITY AGENTS

There are many natural products that have been used for anti obesity agent. Tea polyphenolics like 3-o-gallate show a potent lipase inhibitor activity. 3Methylethergangalin and 5-hydroxy- 7-(4'hydroxy-3'-methoxyphenyl)-1- phenyl-3- heptanone isolated from Alpinia officinarum have shown significant lipase inhibitory action . Garcinia cambogia have hydroxycitric acid which is used as an antiobestiy agent . Guggulipid, a fraction of Commiphora mukul resin and has been developed at CDRI LUCKNOW, and have guggulsterone act as hyperlipidameic agent .

2. ANIMAL SOURCES

Animal have also been a source of some drugs. The skin of an Ecuadorian poison frog is a source of Epibatidine, which is ten time more potent then morphine . Cure of several diseases have been done by venoms and toxins of several animals. Teprotide from the extract of Brazilian viper, has led to the development of cilazapril and captopril, which are effective anti hypertensive drugs .

3. MICROBIAL SOURCES

Microbe as the source novel bioactive agents come under investigation since from the serendipitous discovery of penicillin from the filamentous fungus Penicillium notatum discovered by Fleming in 1929 and got the Nobel prize in 1945. After publication of the first clinical data on penicillin G between 1942–1944, there was a worldwide efforts to discover new antibiotics from microorganisms . Tetracycline is another antibiotic which is obtained from Streptomyces aureofaciens used in UTI , achne and in several dental infections Choramphenicol obtain from Streptomyces venezuelae is another prominent example which is used in typhoid, cholera and in brain abscesses .Further the discovery of novel antibiotic structural classes was done that include the isolation of the antibiotics imipenem norcardicin and aztreonam. Further the macro fungi such as polypores are a large group of wood-rotting fungi of the phylum Basidiomycota (basidomycetes) and Ascomycota, which are a major source of pharmacologically active substances. Polypore fungi have shown strongantimicrobial compounds also have antiviral, cytotoxic, antineoplastic, cardiovascular, anti-inflammatory, immune-stimulating agent . In 1953, Edmund Kornfeld first isolated vancomycin a glycopeptide antibiotic produced in cultures of Amycolatopsis orientalis which is active against a wide range of grampositive organisms such as Staphylococci and Streptococci and against gram-negative bacteria, mycobacteria and fungi. The macrolide erythromycin from Saccharopolyspora erythraea is an antibacterial drug. It has broad spectrum activities against gram-positive cocci and bacilli and is used for respiratory tract infections.

Betulinic acid , a triterpenoid obtained from the bark of Betula pubescens was identified as a weak inhibitor of HIV. Bevirimat , extracted from a Chinese herb Syzygium claviflorum is used to inhibit the final step of the HIV Gag protein processing. Ganoderic acid β , isolated from the fruiting bodies and spores of Ganoderma lucidum, displayed significant antiHIV-1 protease activity. Amrubicin hydrochloride, related to the anthracycline, doxorubicin (Adriamycin®),was isolated from the fungus Streptomyces peucetius. is used to treat acute leukaemia, soft tissue and bone sarcomas, lung cancer, thyroid cancer and both Hodgkins and non-Hodgkins lymphomas .Torreyanic acid was isolated from an endophyte from the endangered tree, Torreya taxifolia and was tested in several cancer cell lines. The salinosporamide A , has been isolated from actinomycete genus named Salinospora has been cultured using appropriate selective isolation techniques, and a very potent cytotoxin and proteasome inhibitor . Ambuic acid is an antifungal agent, which has been recently isolated from of Pestalotiopsis Microspora.

4. MARINE SOURCES

70% of planet earth's surface is covered by ocean, pharmaceutical companies began to realize that the ocean would possess unique biodiversity and may be a possible source for potential drug candidate .These progressive advancements in the past 40 years of exploration of the marine environment have resulted in the isolation of thousands of structurally unique bioactive marine natural products. Some examples include, Ziconotide (Prialt®, Elan Corporation) a peptide first discovered in a tropical cone snail, which was approved for the treatment of pain.

Plitidepsin(Aplidin®,PharmaMa), a depsipeptide was isolated from the Mediterranean Tunicat Aplidium albicans. Plitidepsin is effective intreating various cancers, including melanoma, small cell and non-small

cell lung, bladder as well as non- Hodgkin lymphoma and acute lymphoblastic leukemia. Ecteinascidin 743 (ET743; YondelisTM) was isolated from the ascidian Ecteinascidia turbinate and used as an anticancer agent. Spisulosine , isolated from the marine clam Spisula polynyma, exhibited substantial selective activity against tumor cells compared to normal cells. Cryptophycin recognize cancerous tumor cells, even those of "solid tumors" such as those in brain, colon, ovarian, prostate, pancreas, lung and breast cancers and it can destroy the cells of multi-drug resistant (MDR) tumors .

Natural Products from Marine Algae:Green, brown and red algae have been intensively assessed for their antibacterial and antifungal activities. The brown algae, Dictyota dichotoma afforded diterpenes, , dictyolides A , 4- acetoxydictylolactone dictyolides B and nordictyolide which display antitumor activities . Another example is crenuladial , isolated from the brown alge. Dilophus ligatus which displayedantimicrobial activity against Staphylcoccus aureus, Micrococcus luteus and

Aeromonas hydrophyla .

Natural product discovery Approach

Screening of natural product extracts is complicated due to the presence of fluorescent or insoluble compounds. Advances in detection technologies and new screening assays have overcome many of these challenges. Following are the approaches for drug discovery:

1. CELL-BASED ASSAYS

They are generally preferred in drug discovery because the assessment of molecular interactions occurs within the context of a living cellular environment. In addition, information about drug penetration is obtained early on. However, cell based activities are more variable and less sensitive, and may be more resource intensive due to extensive assay development time. Cell based assays can be simple growth inhibition assays measuring the effect of compounds on cellular growth. Such assays use spectrophotometric or turbidimetric method for detection of activity. Other cell- based assays that are frequently used in natural product discovery are those that measure activation of genes upstream of cellular functions like proliferation and differentiation .

2. BIOCHEMICAL ASSAYS

Biochemical assays have the advantages of providing target-specific information. One of the newer biochemical assays is a capillary electrophoresis (CE) technique used for the detection of functional activity of compounds as well as their relative binding strengths in crude extracts even in the presence of interferences. This approach uses electrophoretically mediated micro-analysis (EMMA) which incorporates laser induced fluorescence detection for maximum detection.

3. NEWER DRUG DISCOVERY

Although bioactivity assays are most commonly employed to identify lead structures, newer screening methods are being developed which do not necessarily depend on an initial understanding of bioactivity. A virtual screening approach used in combination with HTS has proven to be effective in the search of neuraminidase (NA) inhibitors for influenza viruses A and B. The combination of virtual screening with HTS not only saves time but also money . Other non-activity–based lead identification methods have utilized genetic studies of biosynthetic pathways of natural product. Trichamide a cyclic peptide produced by a biosynthetic gene cluster in the genome of the global, bloom forming marine cyanobacterium Trichodesmium erythraeum . Similarly, ECO- 02301 an anti fungal secondary metabolite, was successfully discovered by a genomic approach. Another example is E-837Another example is aspoquinolones A-D, four prenylated quinolin- 2-one alkaloids, were produced from Aspergillus nidulans HKI by the combination of genomic and analytical screening approaches . The benefit of natural products is that their biological sources is most likely available and can be employed for production. With recent advances in whole genome sequencing, it is also likely that the genome of the biological source itself can be sequenced. By growing knowledge of the pathways and developments in genetics and sequencing it is become increasingly possible to manipulate these pathways to generate a new set of biologically active molecules that are similar to the parent compound.

Importance of Plants in modern medicine

Plants can be serving as source of bioactive compounds with new chemical entities of wide structural diversity, which can be used: directly as bioactive compounds, as drug precursors, as drug prototypes, as pharmacological tools and as marker compounds for standardization of extracts.

I- Plant secondary metabolites as drugs

Group	Compounds present	Main Features	Pharmaceutical properties
Alkaloids	Hormones, carotenoid pigments, sterols, latex and essential oils	Group of major importance with more than 40,000 molecules, they are considered of importance for the survival of plants. They are insoluble in water and are derived from the union of isoprene units	Anticarcinogenic, antiulcerous, antimalarial, antimicrobial, etc.
Phenolic compounds	Coumarins, flavonoids, lignin and tannins.	They are derived from a phenol group	Antidiarrheals, antitumorals, antibacterials, antivirals and enzyme inhibitors (Isaza, 2007)
Glycosides	Saponins, cardiac glycosides, cyanogenic glycosides and glucosinolates.	They arise from the condensation of a sugar molecule with another containing a hydroxyl group, thus forming a glycosidic bond	Antimicrobials, fungicides, insecticides, anticancer, anti-inflammatory and allelopathic (Agustín *et al*., 2011)
Terpenes	Quinoline, isoquinoline, indole, tropane, quinolizidine, piperidine, purine, pyrrolizidene.	Group with about 15000 secondary metabolites. They are soluble in water, contain at least one nitrogen atom and exhibit biological activity. Most	At high doses, most are very toxic, however, at low doses they work as muscle relaxants, tranquillizers, antitussives

The second half of the twentieth century witnessed a rapid development in the methods and instruments in the process of plant‘s secondary metabolites isolation. In addition to that, the potential activities of the isolated compounds were understood at a molecular level. Thousands of secondary metabolites were isolated and used as drug such as: Digoxin which used as cardiotonic is isolated from Digitalis purpurea (purple or common foxglove), Aescin which used as anti-inflammatory, venotonic, and as anti- eadematous drug is isolated from Aesculus hippocastanum (horse chestnut). While Ajmalicine the indole alkaloids used for treatment of circulatory disorders was first isolated from Rauwolfia sepentina by Siddiqi in 1931. Cragg and associates conducted an analysis and found out that 30% of the approved drugs by the FDA (Food and Drugs administration) between 1983-1992 were of natural origin.

II. Plant secondary metabolites as drug precursors

The semi-synthetic approach is usually used to resolve the shortage of supply due to the low yield of compounds from plants and/or the high cost of total synthesis. Drug precursors can be converted into the compound of interest by chemical modificationor fermentation methods. The following examples indicate some secondary metabolites from plants are useful drug precursors, although they are not necessarily pharmacologically active in their original naturally occurring forms: Paclitaxel (well- known potent anticancer compound) was semi-synthesized by Bristol-Myers Squibb method since 2002 from 10-deacetylbaccatin III which isolated from Taxus baccata. (Family: Taxaceae).

Diosgenin, a steroidal sapogenin obtained from the tubers of variousDioscoreaspecies can be converted chemically in several steps into progesterone. Oseltamivir phosphate (Tamiflu)® is developed for the treatment and prophylaxis of influenza viruses A and B. The starting material for the oseltamivir synthesis is (−) shikimic acidCurrently, Roche, the drug manufacturer, still relies on both extraction and fermentation methods to obtain ton quantities of shikimic acid.

III. Plant secondary metabolites as drug prototypes

Drug prototype is the first compound discovered in a series of chemically related therapeutic agents. In 1996, from a total of 244 drug prototypes identified in one analysis from minerals, plants, animals, microbes, and chemical sources, plant secondary metabolites contributed 56 of these (23 %). With advances in organic chemistry, medicinal chemists started preparing analogs from these drug prototypes to provide safer and more potent drugs such as: Several anti-neoplastic compounds isolated from plants, such as podophyllotoxin and camptothecin, were too toxic and not water soluble enough for clinical application, and analogs with higher therapeutic indices such as etoposide (Vepesid)® and topotecan have been developed in consequence. Guanidine is a natural product with good hypoglycemic activity isolated from Galega officinalis L., but it was too toxic for clinical use. Many derivatives of guanidine have been synthesized, metformin (dimethylbiguanide) was later found to be clinically suitable for treatment of type II diabetes.

IV. Plant secondary metabolites as pharmacological probes

Pharmacological probes help researchers to understand the mechanism of action of intracellular signal transductions and biological mechanisms related to human disease, which can aid the design of better drugs. Genistein, an isoflavone found naturally in soybean (Glycine max Merr.), is an inhibitor of various protein tyrosine kinases (PTK), which are essential enzymes involved in intracellular signal transduction. Genistein is used to probe the interaction between PTK and cyclic nucleotide-gated (CNG) channels, which are important in mammalian olfactory and visual systems. Phorbol is a tetracyclic diterpenoid plant secondary metabolite isolated as a hydrolysis product of croton oil from the seeds of Croton tiglium L. Various 12, 13-diesters of phorbol have the capacity to act as tumor promoters.

Development of new technologies in pharmaceutical discovery research

1. Combinatorial Chemistry

Combinatorial chemistry generates larger libraries but the compounds therein are relatively simple planar molecules contrary to the natural products' pool that gives a much higher hit-rate in high throughput screening with high chemical diversity. Further, improvements in isolation, purification and characterization procedures have fastened the output of natural product research, thereby reviving the interest of the pharmaceutical industry. Combinatorial chemistry has evolved from its early focus as a random strategy for generating molecular diversity into a powerful design technology for developing and optimizing drug candidates.

Increasing pressure to identify, optimize, develop, and commercialize novel drugs more rapidly and more costeffectively has led to an urgent demand for technologies that can reduce the time to market for new products. Molecular diversity, of both natural and synthetic materials, provides a valuable source of compounds for identifying and optimizing new drug leads. Through the rapidly evolving technology of combinatorial chemistry, it is now possible to produce libraries of small molecules to screen for novel bioactivities. This powerful new technology has begun to help pharmaceutical companies to find new drug candidates quickly, save significant dollars in preclinical development costs, and ultimately change their fundamental approach to drug discovery. Comprising the work of the leading authorities in the area of molecular diversity and combinatorial chemistry, combinatorial chemistry and molecular diversity in drug discovery highlights the critical concepts and issues involved in implementing combinatorial chemistry to chemical libraries.

As above mentioned, combinatorial chemistry was a The Success of Natural Products in Drug Discovery 25 key technology enabling the efficient generation of large screening libraries for the needs of high-throughput screening. However, now, after two decades of combinatorial chemistry, it has been pointed out that despite the increased efficiency in chemical synthesis, no increase in lead or drug candidates have been reached. This has led to analysis of chemical characteristics of combinatorial chemistry products, compared to existing drugs and/or natural products. The chemo-

informatics concept chemical diversity, depicted as distribution of compounds in the chemical space based on their physicochemical characteristics, is often used to describe the difference between the combinatorial

chemistry libraries and natural products. The synthetic, combinatorial library compounds seem to cover only a limited and quite uniform chemical space, whereas existing drugs and particularly natural products, exhibit much greater chemical diversity, distributing more evenly to the chemical space. The most prominent differences between natural products and compounds in combinatorial chemistry libraries are the number of chiral centers (much higher in natural compounds), structure rigidity (higher in natural compounds) and number of aromatic moieties (higher in combinatorial chemistry libraries).

2. High-Throughput Screening

High-Throughput Screening (HTS) is a standard method for hit discovery for scientific experimentation especially used in drug discovery and relevant to the fields of biology and chemistry . Using robotics, data processing and control software, liquid handling devices, and sensitive detectors, HTS allows a researcher to quickly conduct millions of biochemical, genetic or pharmacological tests. Through this process one can rapidly identify active compounds, antibodies or genes which modulate a particular biomolecular pathway. The results of these experiments provide starting points for drug design and for understanding the interaction or role of a particular biochemical process in biology. In essence, HTS uses a brute-force approach to collect a large amount of experimental data—usually observations about how some biological entity reacts to exposure to various chemical compounds—in a relatively short time. A screen, in this context, is the larger experiment, with a single goal (usually testing a scientific hypothesis), to which all this data may subsequently be applied. A screening facility typically holds a library of stock plates, whose contents are carefully catalogued, and each of which may have been created by the lab or obtained from a commercial source.

These stock plates themselves are not directly used in experiments; instead, separate assay plates are created as needed. An assay plate is simply a copy of a stock plate, created by pipetteing a small amount of liquid (often measured in nanoliters) from the wells of a stock plate to the corresponding wells of a completely empty plate.

Automation is an important element in HTS's usefulness. A specialized robot is often responsible for much of the process over the lifetime of a single assay plate, from creation through final analysis. An HTS robot can usually prepare and analyze many plates simultaneously, further speeding the data-collection process. HTS robots currently exist which can test up to 100,000 compounds per day. HTS is a relatively recent innovation, made lately feasiblethrough modern advances in robotics and highspeed computer technology. It still takes a highly specialized and expensive screening lab to run an HTS operation, however, so in many cases a small-to- moderately sized research institution will use the services of an existing HTS facility rather than set up one for it.

According to Dietzman, when using natural products to support High Throughput Screening (HTS), discovery programs must include effective technologies to support the acquisition and inventory of the natural products sources. A critical element in this process is information handling. Information handling requirements at every stage of this process will grow, and computer technology is available to meet the demand. With the advance of screening technologies, it is now possible to evaluate upwards of 100,000 test substances per week against several different targets. The required test substance quantities are as low as a

few micrograms per assay. In keeping with these trends, natural products acquisition programs are now scoped to collect large numbers of diverse tissue samples, but only to collect small wet weights. This format allows collectors to provide large numbers of samples for screening, and they rely on recollection for follow-on studies. This means that discovery programs actively testing natural products must track a greater number of samples. In addition, information on known natural products chemistry must be considered in an effort to contain the costs of follow-on studies. When a natural product registers as a confirmed "hit" in a discovery screen, the people involved simply want to know: what is it, where did it come from, is it novel and how to get more. The general information handling system requirements, therefore, fall

into two categories: recollection, and dereplication. Recollection must be a cornerstone of any natural products acquisition program; you must be able to go back and get more of the source organism for follow-on studies. Dereplication against the growing number of known chemical compounds is becoming increasingly important for discovery screens. Based upon initial chemical characterization of an active fraction(s), a chemical substructure-based database search can be performed to compare with, and dereplicate against, known chemical compounds.

Laboratory chemists are at a tremendous advantage when they can generate a list of possible chemical structures to compare with an extract containing unknown compounds. A chemist can then quickly evaluate the possibility for the presence of known compounds and will be able to make an informed decision regarding the interest in the extract as a source of novel bioactive compounds. This rapid process can save the costs of follow-on isolation and structural elucidation studies and increase a program's efficiency.

3. Bioinformatics, Proteomics and Genomics

Proteomics include technologies for protein mapping (separating, distinguishing and quantifying the proteins present in individual samples), and techniques for identifying specific proteins and characterizing their complete structure and functional role. The main protein mapping technology in use today is two-dimensional polyacrylamide gel electrophoresis (2D-PAGE), which can resolve up to 2000 proteins on a single gel— significantly better than other separation techniques, but still not the 6000 proteins expressed by typical tissues. According to Harvey, with recent technological developments in molecular biology, instrumentation and information technology, screening of compounds can be conducted at throughputs that could not have been imagined, even a few years ago.

The availability of molecular targets, the ability to engineer such targets into simple reporter systems such as yeasts, and the use of robotics to handle the samples and conduct the assays make random screening of chemical diversity a very attractive approach to the discovery of novel activity. The techniques of molecular cloning provide the possibility of deriving an understanding of physiological processes at the molecular level. Currently, over 250 gene products relating to major neurotransmitters are known, and hundreds of different subtypes of ion channels have also been characterized genetically. There has been a similar increase in the understanding of intracellular signaling pathways, opening up the possibility of many new target sites for drugs. The drug discovery process is becoming more and more complex and capital-intensive, and such companies remain "target rich" but "lead poor", with lead discovery as a greater bottleneck.

In such a situation, industrialization of drug discovery process is underway. Although highthroughput screening (HTS) and combinatorial chemical synthesis are explored with great hope, general experience tells us that in most companies the investments in these technologies have not reaped rewards in new lead discovery as expected. Powerful new technologies such as HTS and combinatorial chemistry are revolutionizing drug discovery. But natural products still offer unmatched structural variety, especially as new environmental niches are explored, and their usefulness can be further extended by engineering the proteins that produce them and using them to

probe biological pathways.

On the other hand, the sequencing of the human genome and numerous pathogen genomes has resulted in an unprecedented opportunity for discovering potential new drug targets. Chemogenomics has appeared as a new technology to initiate target discovery by using active compounds as probes to characterize proteome functions. Natural products are the ideal probes for such research. Binding affinity fingerprint is a powerful chemogenomic descriptor to characterize both small molecules and pharmacologically relevant proteins .

The successful of natural product in drug discovery

1. Screening and Design

Advancement in the knowledge of molecular mechanisms, cellular biology and genomics not only increased the number of molecular targets but also demanded shorter drug discovery timelines. The advent of newer combinatorial techniques of synthesis and computational methods, with the incongruity of natural products to keep pace with the ever growing competition for novel classes of drugs at a faster rate, has prompted pharmaceutical industries to look towards synthetic chemical libraries.

Though pharmaceutical industry appreciates the role of nature as the chief architect of natural products' libraries and respect the science therein, they fear carrying out research in the area. However, the rethinking on fresh strategies is on the verge of gaining prominence due to disappointing results of combinatorial chemistry and high throughput screening (HTS) in delivering potent chemical entities.

The old laborious processes involved in the extraction and isolation were not capable of generating the numbers which were required to keep pace with the HTS requirements. Actinomycetes and fungi are used as screening sources by all or almost all organizations that conduct natural product-based drug discovery (NPDD) programs.

Bacteria, plants, and medicinal herbs come next. Some apply invertebrates and microalgae to screening. Insects are rarely exploited. But some seek microbes from unusual environments. Since initial natural products are rarely launched unaltered as a drug, unreasonable expectations on financial investment will prevent many countries from being involved in the drug discovery process. Pharmacognosy provides the tools to identify select and process natural products destined for medicinal use.Usually, the natural product compound has some form of biological activity and that compound is known as the active principle-such a structure can act as a lead compound (not to be confused with compounds containing the element lead). Many of today’s medicines are obtained directly from a natural source.

The process of finding a new drug against a chosen target for a particular disease usually involves HTS, where large libraries of chemicals are tested for their ability to modify the target. Another important function of HTS is to show how selective the compounds are for the chosen target. The ideal is to find a molecule which will interfere with only the chosen target, but not other, related targets. To this end, other screening runs will be made to see whether the "hits" against the chosen target will interfere with other related targets- this is the process of cross-screening. Cross-screening is important, because the more unrelated targets a compound hits, the more likely that off-target toxicity will occur with that compound once it reaches the clinic. It is very unlikely that a perfect drug candidate will emerge from these early screening runs. It is more often observed that several compounds are found to have some degree of activity, and if these compounds share common chemical features, one or more pharmacophores can then be developed. While HTS is a commonly used method for novel drug discovery, it is not the only method. It is often possible to start from a molecule which already has some of the desired properties. Such a molecule might be extracted from a natural product or even be a drug on the market which could be improved upon. Other methods, such as virtual high throughput screening, where screening is done using computer-generated models and attempting to "dock" virtual libraries to a target, are also often used. Another important method for drug discovery is drug design, whereby the biological and physical properties of the target are studied, and a prediction is made of the sorts of chemicals that might fit into an active site. Novel pharmacophores can emerge very rapidly from these exercises. Once a lead compound series has been established with sufficient target potency and selectivity and favorable drug-like properties, one or two compounds will then be proposed for drug development. The best of these is generally called the lead compound, while the other will be designated as the "backup". Combinatorial chemistry may simply have increased the volume of haystack in which the needle is hidden. Natural compounds certainly have biological activity in their original locations. However, natural product research needs long and deep experience, particularly in taxonomy, which cannot be built up in one day. If this know-how is abandoned, it will never be recovered in the future.

One of the solutions will be out-sourcing the activity, because a number of technologically advanced biotech ventures will play a key role of NPDD. The modern natural product research is undergoing a revolution due to recent advancements in combinatorial biosynthesis, microbial genomics and screening processes. Moreover, access to hyphenated techniques like Liquid Chromatography-Mass Spectrometry, Liquid Chromatography-Nuclear Magnetic Resonance have raised the hope of drastically reducing the time and cost involved in natural product research by using dereplication processes that are combination of techniques to avoid the already reported compounds. The unique molecular diversity of natural compounds can be leveraged in the design of combinatorial libraries to improve their inherent biological activity or drug-like properties.

This can be performed by semi-synthetic modification of the parent molecule or by fully synthetic methods after crucial structural elements required for biological activity are defined from the libraries and refined by computer-assisted drug designing methods. In comparison to the past, more specific targets, efficient in vitro as well as in vivo models are available and can be utilized. Current strategies in natural product research involve a multifaceted approach combining botanical, phytochemicals, biological and molecular techniques. In a paradigm shift from discovery of single bioactive molecules, multi-constituent mainstay of bioactive

extracts can be emphasized for synergistic and antagonistic studies at cellular and molecular levels. Of the 250,000 species of higher terrestrial plants in existence, only 5 to 15 percent are estimated to have been chemically and pharmacologically investigated in systematic fashion. Today, the perceivable threat of extinction of biodiversity due to global warming and other environmental reasons is also very high.

Thus, there is an urgent need to identify the indigenous natural resources to study them in detail for use in drug discovery. Moreover, sustainable supply of medicinal plants is essential for practicing traditional medicine as well as natural product drug discovery.

The pharmaceutical industry has to come forward in utilizing the knowledge available in traditional medicines. These later may provide cure for different types of diseases and disorders but need scientific validation. In order to focus the research on traditional medicines to serve national interests, the first priority is to assess the therapeutic quality of herbal medicines objectively since the dividing line between the modern therapies and traditional therapies remains imprecise. The assessment of quality can be made easier if distinction is drawn between rational and empirical medication taking into account marked psychodynamic effects associated not only with the active substances, but also with their typical indications. It is satisfying to note that companies have started their efforts for using the traditional knowledge in the development of their formulations as well as in drug discovery programs. The formulations that will be manufactured in these companies use Good Manufacturing Practices and Best Quality Practices to ensure standardized, safe and effective herbs as well as finished phytomedicines that can be made popular like the Chinese medicines all over the world. The therapeutic areas of inflammation, metabolic diseases and obesity may receive more attention for the development of natural products. Inflammation process is considered to be the root cause of almost all the diseases including cancer at the molecular level. Lifestyle diseases like obesity and metabolic disorders are silent

2. Chemical Diversity of Natural Products

It is not uncommon for natural products to have complex molecular structures, with cyclic semi-rigid scaffolds, several chiral centers, more than five H-bond donors, more than ten H-bond acceptors, more than five rotatable C-C bonds, a large polar surface area, and a molecular weight above 500. While this may lead to moderate levels of bioavailability and corresponding dosage regimens to achieve the required efficacy, there may be a latent advantage in such complex structures, as has been previously hypothesized.

This complexity has occasionally been thought to be a constraint for the total chemical synthesis of natural products-based libraries in a given time and resource frame. Chemical diversity in nature is based on biological and geographical diversity, so researchers travel around the world obtaining samples to analyze and evaluate in drug discovery screens or bioassays. This effort to search for natural products is known as bioprospecting. On the other hand, some medicines are developed from a lead compound originally obtained from a natural source. This means the lead compound can be produced by total synthesis, or can be a starting point (precursor) for a semi-synthetic compound, or can act as a template for a structurally different total synthetic compound. This is because most biologically active natural product compounds are secondary metabolites with very complex structures. This has an advantage in that they are extremely novel compounds but this complexity also makes many lead compounds' synthesis difficult and the compound usually has to

be extracted from its natural source-a slow, expensive and inefficient process. As a result, there is usually an advantage in designing simpler analogues.

Historically, most drugs have been derived from natural products, but there has been a shift away from their use with the increasing predominance of molecular approaches to drug discovery. Nevertheless, their structural diversity makes them a valuable source of novel lead compounds against newly discovered therapeutic targets. Technical advances in analytical techniques mean that the use of natural products is easier than before. It has long been recognized that natural-product structures have the characteristics of high chemical diversity, biochemical specificity and other molecular properties that make them favorable as lead structures for drug discovery, and which serve to differentiate them from libraries of synthetic and combinatorial compounds.

Moreover, natural products typically have a greater number of chiral centers and increased steric complexity than either synthetic drugs or combinatorial libraries . In addition, natural products differ significantly from synthetic drugs and combinatorial libraries in the ratio of aromatic ring atoms to total heavy atoms (lower in natural products), number of solvated hydrogen-bond donors and acceptors (higherin natural products) and by greater molecular rigidity. Natural-product libraries also have a broader distribution of molecular properties such as molecular mass, octanol water partition coefficient and diversity of ring systems compared with synthetic and combinatorial counterparts. Indeed, less than one-fifth of the ring systems found in natural products are represented in current trade drugs .

3. Isolation and Purification

If the lead compound (or active principle) is present in a mixture of other compounds from a natural source, it has to be isolated and purified. The ease with which the active principle can be isolated and purified depends very much on the structure, stability, and quantity of the compound. In past, it was not possible until the development of new experimental procedures such as freeze drying and chromatography that the successful isolation and purification of penicillin and other natural products became feasible.

4. Identification of Biologically Active Material

Two main approaches exist for the finding of new bioactive chemical entities from natural sources; random collection and screening of material, or exploitation of ethnopharmacological knowledge in the selection. The former approach bases itself on the fact that only a very small part of globes' biodiversity has ever been tested for any biological activity, and on the other hand, particularly organisms living in a species-rich environment need to evolve defense and competition mechanism to survive. Thus, collection of plant, animal and microbial samples from rich ecosystems may give rise to isolation of novel biological activities. One example of a successful use of this strategy is the screening for antitumor agents, performed by National Cancer Institute in USA started in 1960s. Cytostate paclitaxel (taxoid) was identified during this campaign from Pacific yew tree Taxus brevifolia. Paclitaxel showed antitumor activity with previously unknown mechanism (stabilization of microtubules) and is now approved for clinical use for the treatment of lung, breast and ovary cancer, as well as for Kapos sarcoma. Material may be done by collecting knowledge on use of plants and other natural products as herbal medicines and thereby get an idea of potential biological

activities. It is worth remembering that a major part of earth's population still relies on nature-derived drugs as their only medication. Ethnobotany, the study of the use of plants in the society, and particularly ethnopharmacology, an area inside ethnobotany focused on medical use of plants, may therefore provide invaluable information, as illus trated by the example of artemisinin, an antimalarial agent from sweet wormtree Artemisiae annua, used in Chinese medicine since 200 DC and nowadays in use against multiresistant malarial protozoa Plasmodium falsiparum.

5. Structure- ctivity and Structure-Property Relationships of Natural Products

One of the distinguishing features of natural products is their frequent occurrence as suites or complexes of structurally related analogues. The biological significance of this expressed molecular diversity is unclear, particularly when the suite contains members (often major) that seem to lack biological activity or function. Essentially, why would an organism expend the resources needed to synthesize many analogues of a molecule for which there is but a single purpose? One possible answer to this question is furnished by the "screening hypothesis", which is based on the proposition that biological activity is a rare property for any molecule to possess, and that there might be a selective advantage to an organism if it can generate and retain chemical diversity at a low cost. The existence of congeners of a natural product series might therefore be the consequence of an organism's need to generate its own chemical diversity to optimize the activity of its secondary metabolites, essentially doing its own structure–activity relationship (SAR) optimization .

6. Development of New Technologies in Modern Methods of Drug Discovery

The success of drugs derived from nature added a new driving force to screening for novel, biologically active metabolites from these products. Since the 90s, new Pharmaceutical Discovery Research: Technologies such as combinatorial chemistry, high throughput screening, bioinformatics, proteomics and genomics have emerged, and are being integrated widely in the field of pharmaceutical discovery research. These technologies have enormous potential to make use of the chemical diversity of natural products. Other techniques have only been developed in the last years and continue to evolve rapidly. These include molecular diversity, compound library design, protein 3D-structures, NMRbased screening, 3D QSAR in modern drug design, physicochemical concepts, and computer-aided prediction of drug toxicity and metabolism. During that period, technologies for drug discovery advanced and diversified greatly. NPDD activities compete rather strongly with HTS, combinatorial chemistry and genomics. New approaches to improve and accelerate the joint drug discovery and development processes are expected to take place mainly from the innovation in drug target elucidation and lead structure discovery. Powerful new technologies are revolutionizing drug discovery. When using natural products to support HTS, discovery programs must include effective technologies to support the acquisition and inventory of the natural products sources. A critical element in this process is information handling. Information handling requirements at every stage of this process will grow, and computer technology is available to meet the demand. With the advance of screening technologies, it is now possible to evaluate upwards of 100,000 test substances per week against several different targets. The required test substance quantities are as low as a few micrograms per assay. In keeping with these trends, natural products acquisition programs are now scoped to collect large numbers of diverse tissue samples, but

only to collect small wet weights. This format allows collectors to provide large numbers of samples for screening, and they rely on recollection for follow-on studies.

Conclusion

Natural products as building blocks for molecular libraries, Instead of viewing natural products as a stand-alone approach distinct from combinatorial synthesis, it is now much more effective to implement strategies that combine both approaches. In various principle, it is seems the unique molecular diversity of natural products can be leveraged in the design of combinatorial libraries. The target-oriented or focused- library approach seeks to elaborate structural modifications onto an existing bioactive naturalproduct scaffold in analogue patterns, systematic fashion in order to ameliorate its inherent biological activity or drug-like properties.

Presently, the drug discovery engine operates at an accelerated pace in comparison with the era in which natural products were pre-eminent sources of drug leads, numerous approaches have been developed to capture their intrinsic value. The essential breakthroughs in separation and structure determination technologies have lowered the hurdles inherent in screening mixtures of structurally complex molecules.

The confluence of these technologies with advances in genomics, metabolic engineering and chemical synthesis offer the new method along with the technologies to explore the remarkable chemical diversity of nature's 'small molecules'.

Future Prospect

Combinatorial chemistry may simply have increased the volume of haystack in which the needle is hidden. Natural compounds certainly have biological activity in their original locations. However, natural product research needs long and deep experience, particularly in taxonomy, which cannot be built up in one day. If this knowhow is abandoned, it will never be recovered in the future. One of the solutions will be out-sourcing the activity, because a number of technologically advanced biotech ventures will play a key role of NPDD. Biodiversity and genomics are two key words governing bioscience in the 21st century.

Biological diversity is increasingly important in this field. Industries need to adapt to the new regulations and have clear visions of what their needs are for biological resources as tools for innovation. On the other hand, organizations with title and access to resources need to find ways to facilitate sustainable use of their biological resources if they really want to participate in and benefit from the drug discovery process. Everyone should understand that even though abiological resource

may have great intrinsic value as a component of an ecosystem and a reservoir of genetic information, its monetary value might be minimal until a utility is discovered and developed. To realize that utility and to share in the benefits, organizations should be prepared not only to participate actively in the discovery process, but also to share the financial risks.

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