Tailoring Biodiversity for Development of New Therapeutics

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Abstract

The nature remains as the potential source of organic structures of unparalleled diversity. Successful drug discovery process is often a function of the diversity of chemotypes examined, and therefore, drug discovery programs must aim to evaluate broadest diversity of chemical classes from natural resources in minimum samples and least time. An evaluation of the ecological/environmental ramifications of populations for screening operations and pre-development studies is an important facet for finding new chemotypes. Historically plants and microorganisms have been extraordinary sources of bioactive compounds and further continue to do so with the recent development of advanced techniques and tools viz., high throughput screening systems, combinatorial chemistry and genomics for isolation, characterization and establishing the structure–activity relationships of the biological extracts as well pure compounds. Many new companies have been setup in last few years to explore natural products using techniques like combinatorial biosynthesis and gene transfer possibilities for identification of novel substances heretofore unaccessible for testing. It is critical that biomass diversity be maintained to provide future structural diversity, leads and drugs for pharmaceutical targets that will emerge in the coming years.

Key words: Biodiversity, drug discovery, natural products.

Introduction

Biodiversity throughout the world is of prime importance to the human population and indeed the stability of the entire world. A vast genetic variety available in terrestrial plants, animals and microorganism offers us a wealth of possibilities for the production of new pharmaceuticals, nutraceuticals and biomaterials. The intention of the present paper is to prospect the biodiversity for the modern pharmaceutical industry. Secondary metabolites often have an important function and are generally produced by plants, animals and microorganisms for specific reasons. These privileged structures so designed by the nature interact with the biological macromolecules like the proteins, DNA and modulate their function thereby exhibiting a variety of activities.

The history of man using plant and plant products dates back many thousand of years to the time when vegetables were the only source of therapeutics. Historically, teas and extracts
were derived from natural sources through trial and error and have some efficacy to treat human disease. Such experiences collected by shamen and healers persisted and subsequently with minor improvements were developed and have been documented in Chinese, Ayurvedic, European and African systems of medicine. The use of traditional medicine and medicinal plants in most developing countries as a normative basis for the maintenance of good health has been widely observed (UNESCO, 1996). This system of traditional medicine is gaining popularity as Over the Counter (OTC) polyherbal preparations whereas the industrialized western societies have a different approach in using this knowledge by isolation and characterization of potentially active components, establishing the structure activity relationship for developing new entities to be used as a variety of pharmacological agents.

Approximately 25% of the active components currently used have their origin from flowering plants and this share is going to increase to around 35% in the next five years with the demand from consumers for natural based medicines (Ortega, 1998). Pharmaceutical industries have renewed their interest in potential discovery of new compounds from plants, and are in line with the increased awareness and interest in medicinal plants and natural treatments, in both general public and the scientific community. The 1990's saw around 125 top pharmaceutical companies introducing the natural products research into their research programs.

Natural to chemical diversity
Nature can be considered as the ultimate chemist as natural products offer us with an abundant source of novel chemo-types, pharmacophores or lead structures, which could be directly used or derived into ready-made drugs. Natural product chemists and phytochemists recognize biological matrices like plants and microbes to

produce a bewildering diversity of secondary metabolites.

The wide variety of compounds, originating from plants and microbes can be used directly or indirectly in modern medicine today. Mevacor®, a proprietary drug for hypercholesterolemia was isolated from fermentation broth of Monascus ruber and Aspergillus terreus Thom. (both fungi) at Merck R&D in early 1980’s. Cyclosporin initially discovered at Sandoz R&D from Tolypocladium inflatum is being currently marketed by Novartis as Sandimmune® as the major transplantation surgery drug, are the examples of unmodified natural drugs (Young, 1999; Borel & Kis, 1991). Apart from traditionally used digitalin from Foxglove some prominent drugs from plant origin include, Artemisin (Artemisia annua Linn.) for treatment of malaria, Vincristine and Vinblastine from Catharanthus roseus (Linn.) G. Don (Periwinkle) for the treatment of cancer and Taxol from European Yew (Taxus brevifolia Nutt.) for treating ovarian and lung cancer. An efficient version of Artemisin, Paluther using combinatorial techniques has been developed by Aventis. Recently a synthetic analog of Taxol called Taxotere® by Rhone-Poulenc has been introduced for refractory breast cancer. Another excellent development on natural product template was development of Maxalt® (rizatriptan) an anti-migraine drug on the basis of mode of action of
ergotamine, a potent serotonin (5-HT) receptor. Maxalt has an improved side effect profile and faster onset of action as compared to ergotamine (Zhang et al., 1996). (Fig 1)

As receptor and enzyme targets have become more available through cloning and expression systems many improved drugs have been obtained from natural products and have served as the source of synthetic medicinal chemistry. At times natural products lack optimal pharmacological properties necessary to be viable in the current modern drug environment, thus a rational modification is carried out to bring in requisite pharmacological properties to develop into a drug. Caspofungin was derived from a complex polypeptide anti-fungal compound pneumocandin B which was discovered from a fungus Glarea lozoyensis in 1985. This compound exhibited appreciable activity against Candida. Analog of echinochandins were prepared and screened for properties like improved potency, stability and water solubility. One such analog L-733560 was finally optimized by the Merck R&D to into a tribasic analog Caspofungin (or Cancidas™). It has been under advanced clinical trials and has shown efficacy in a variety of esophageal and oropharyngeal fungal infections (Ribowicz & Turner, 2002).

So, the natural resources can be anticipated to continue to contribute in the drug discovery efforts by pharmaceutical companies for the foreseeable future.

Selection of biological matrices for screening leads

One of the crucial factors during drug discovery programs is the “selection strategy” adopted to screen a biological matrix for screening the structural biodiversity for use as a drug development candidate. Plant and microbes offer us with a lucrative diversity for screening of the novel bioactives.

Figure 1: Natural and derived structures from biodiversity currently used as drugs.
Microbes as sources of new drug leads

The screening of the fermentative broths of microorganisms began with the discovery of the wonder drug "Penicillin" by Alexander Fleming (1929). A variety of pharmacologically active compounds ranging from antibiotics, immunosuppressants and lipid lowering drugs have been isolated from bacteria and fungi and brought into use since then. The drug discovery groups targeted microbes because of the diversity of species and ease of culture of the microorganisms. Moreover, it is possible to culture and manipulate microorganisms to enhance the production of the wanted compounds.

Microbial diversity is enormous and these have been partially investigated. It has been estimated that only 5000 bacteria have been identified out of a possible 40,000 total and about 70,000 fungi are known of a possible 1500,000 total. So far only 4000 fungal metabolites are described (Dreyfuss & Chapela, 1994) and only 5000-7000 taxonomic species have been studied with respect to their chemistry (Hawksworth, 1991). The samples are generally obtained from diverse sources including soil, dung, leaf litter, on plants, animals, insects and water samples using a variety of fermentation techniques.

However, from the ecological viewpoint there is a need to screen those organisms, which have adapted themselves to the stressful environment. Are the microbes under stressful conditions surviving by an altered metabolism thereby producing novel structures? This needs to be addressed. Fungal sclerotia are essentially vegetative reproducing structures formed under nutritional / environmental stress. These resting structures essentially are bundles of hyphae and differ from the normal mycelium. A group of workers at the natural products division of Merck Laboratories at Rahway, New Jersey have recently highlighted that bioactives produced by these structures are different from the mycelium. They have modelled conditions for the in vitro production of sclerotia by Penicillium group and then screened them for bioactive compounds.

Endophytic microbes are also appealing sources of natural products. These organisms generally reside within the tissues between and among living plant cells. The relationship they establish with the plant varies from symbiotic to pathogenic. Thus, the functional diversity of microbes residing in the plant species is yet to be screened for their bioactive compounds. Taxomyces andreanae, a fungal endophyte, was isolated from the phloem (inner bark) of the Pacific yew, Taxus brevifolia. The fungus is hyphomyceteous and, when grown in a semi-synthetic liquid medium, produced taxol and related compounds (Stierle et al., 1993). The production of taxol by Taxomyces has raised numerous questions about the evolutionary change—is it adaptation or horizontal gene transfer?

Marine organisms also play important host to a variety of microbes and screening of marine microbes for bioactive compounds is gaining popularity among the natural product researchers and drug discovery groups. The approach is based on the assumption that certain physical and biological conditions favour production of diverse range of secondary metabolites. This emphasizes the opportunities of investigating other ecological niches for new bioactives. Marine fungi are shown to be a tremendous source of new biologically active secondary metabolites (Pietra, 1997; Biabani & Laatsch, 1998). Cephalosporin, the major antibacterial antibiotic was isolated from the marine isolate of Cephalosporium sp. (Flynn, 1972). Recently a fraction has been obtained from a bacterium, Alteromonas rubra from the shores of Ohau and contains substances, which are effectively inhibiting the multidrug resistant pathogens: methicillin resistant Staphylococcus aureus (MRSA) and Vancomycin resistant enterococci (VRE) (Gauthier, 1976, Michelle and Hemscheidt, 2000).

Bioprospecting microbes from diverse physical and physiological conditions still remains green area as it can provide us with fascinating structures, which could be directly or indirectly used as therapeutic agents.

Plants as sources of new drugs

Medicinal plants are an integral component of research and development in the pharmaceutical industry with a research focus on isolation and direct use of active medicinal constituents or on the development of semi-synthetic drugs or still again on the active screening of natural products to yield synthetic pharmacologically active compounds. It might appear that most of the plant kingdom has been thoroughly screened for biologically active molecules. However, this is unlikely to be the case. Of the estimated 250,000 species of plants occurring worldwide probably 10% have been scratched for some type of biological activity.
Plants face intense competition for resources and nutrients. At the same time they are under constant attack by viruses, fungi, insects, and predators (pests). For survival, they have to develop an extraordinary array of defense against these pests by way of synthesis of secondary metabolites, most of them being new chemicals. A variety of phytoalexins (naturally occurring antifungal/antibacterial metabolites) have been researched for their crop protection abilities and relatively little work on medical applications has been carried out. Tropical floras contain most of the plant species and it has been estimated that half of these are unknown and never been surveyed for their chemical constituents (Balik, 1990). The alarming rate of the disappearance of tropical flora due to environmental and geological instabilities is responsible for disappearance of a variety of structural templates, yet to be discovered. This has attracted attention of natural products researchers for screening bioactive compounds from tropical plant species for novel chemotypes. Temperate plants still continue to be a source of commercially interesting compounds like Etoposide—a semisynthetic anti-neoplastic agent derived from Mayapple (Podophyllum peltatum) useful in treatment of refractory testicular carcinomas, small cell lung carcinomas, nonlymphocytic leukemias. Atarcurium sesylate is a skeletal muscle relaxant derived from a plant structurally and pharmacologically related to curare.

The fine roots (rhizosphere) of plants are also exposed to a variety of stresses like water, nutrients, desirable microbes and at the same time defend themselves against harmful bacteria, fungi, protozoa, nematodes, insects and other animals. This defense mechanism is entirely chemical and the secondary metabolites secreted by roots in this zone have largely been neglected for screening the bioactive compounds under different environmental conditions. The biodiversity of plant species, coupled with the chemical diversity found within each plant, leads one to the conclusion that plants are perhaps the most valuable source of new bioactive chemical entities. In a recent report it has been highlighted that market of plant derived drugs has touched US$ 30.7 million sales with an annual growth rate of 6.3% since 1997 (Wilkinson, 2000).

Only a small fraction of plants have been systematically investigated for the presence of bioactive compounds. Thus a single plant can serve as a source for a variety of chemical structures having different pharmacological indications.

The crucial factors responsible for ultimate success during the investigation of bioactive constituents for drug designing are the selection of the adequate biological matrix (Plant/microbe), based on the database, the target activity and the assay platform used for establishing the molecule-receptor relationship.

**Biodiversity and drug discovery: Indian scenario**

India is one of the 12 mega biodiversity centers having about 10% of the world's biodiversity wealth, which is divided into 16 agroclimatic zones, 10 vegetative zones and 15 biotic provinces. India is ranked among the major exporters of medicinal plants and vegetative/sap extracts, but when compared to developed countries it stands nowhere with regard to the export of more specific products i.e. the bioactives like alkaloids, hormones, glycosides, etc. in the world.
market (Exim Bank report, 1997). Most of the plant-based raw materials originate in the tropics, but a majority of finished products are manufactured in Europe and North America. Our industry has not grown substantially during last few decades in terms of discovering new molecules from natural products for use as drugs as is being aggressively pursued in the west. Our approach is limited to biological extracts and Ayurvedic preparations and not to the bioactive molecules for establishing the molecule-receptor relationship, which forms the basis of drug discovery and design in the modern pharmaceutical industry. Thus there is an increasing demand for isolating new bioactive molecules. The gravity of the situation has more serious implications in post WTO era.

The need of the hour is to realize the potential of biodiversity and to exploit it fully and judiciously. This envisages selection of the natural resources, isolation, characterization of their bioactive compounds and improvement of potential compounds by using an array of techniques employed in the western countries for improving their bioefficacy for desired pharmacological actions. Central Drug Research Institute (CDRI), Council of Scientific & Industrial Research (CSIR), India has recently taken initiatives in the natural products research. Currently, CDRI is the nodal agency of the all India coordinated programme on development and commercialization of the bioactive substances from plants. This programme involves 22 laboratories out of which 14 laboratories are involved in identifying, collecting and extracting plants based on Ayurvedic/ traditional knowledge while 8 laboratories are engaged in screening and further exploitation of the natural product leads. CDRI is also credited in development of ‘Memory Plus’ (herbal formulation) for memory enhancement derived from the plant Bacopa monnieri (Linn.) Pennell (Brahmi) currently marketed by Nivaran Herbals Private Limited. Cipla is marketing ‘Gulip’ a standardized extract of Commiphora mukul (Hook. ex Stocks) Engl. as a hypolipidaemic formulation developed by CDRI. One of the recent achievements of CDRI is development of ‘Arteether’ — an antimalarial drug derived from Artemisia annua Linn., currently marketed by Themis Chemical Pvt Ltd under the name E- Mal. Neem, Azadirachta indica A. Juss. seeds have been used for the development of certain contraceptives. Dr G.P. Talwar and his group has developed a polyherbal cream using seeds of neem (Talwar, G.P. et al!, 1993).

Several new lead molecules are being identified from hepatoprotective, cardiotonic, wound healing and anti-cancer activities by CDRI. Recently the Indian Pharma Major, Ranbaxy have also initiated research in the area of new drug discovery from natural products in their state-of-art center at Gurgaon.

Natural product drug discovery is essentially “not a one man show” but an integrative multidisciplinary effort channelising the inputs from a botanist, microbiologist to a synthetic chemist and pharmacologist for bringing out relevant molecules out of the biodiversity. The most important facet of this approach is networking of the drug discovery groups with the academia, government and with other drug discovery groups (companies) for accessing diverse natural product resources.
Natural Products & Drug Discovery today - a new perspective

Shortening the time to market and increase the chances of success are the two major issues addressed in development of new pharmaceutical candidates. High throughput screening system has brought in a revolution in drug discovery today. It is an automated tool for testing a variety of extracts in small quantities for a particular activity. Thus random screening of natural extracts from different sources can be carried out using small quantity of raw material, less time and with more accuracy (Lahana, 1999).

Modern drug discovery is based on the molecular-receptor relationship, commonly referred as quantitative structure - activity relationship. It is very rare when a natural product is developed as such and generally must be modified to improve pharmaceutical and biological properties or to reduce the toxicity. Statistics show that within a span of a decade from 1988 to 1994, only 5% drugs were natural products and about 35% were either compounds derived directly from natural products or synthetics based on natural products (Cragg et al, 1997).

There are two basic approaches to drug discovery: rational drug design and traditional method of random screening. Rational design refers to engineering new drug molecules from scratch with the help of computers and molecular biology - requires the knowledge of drug target (such as a receptor or enzyme). So far rational drug designing has limited payoff although it has promising potential.

Combinatorial chemistry has spawned a wave of in-house pharmaceutical research. Combinatorial chemistry shifts compound design to develop efficient versions of a known compound products, by changing one molecule at a time to automated parallel synthesis. The resulting diversity generated boosts the chance to obtain a new compound with better pharmacological properties than their counterparts by reacting efficiently with the disease-causing molecule. Eli Lilly has adopted this technique to optimize existing leads found via traditional medicinal chemistry (Brown, 1996). Development of advanced techniques of purification and characterization has enhanced the area of natural product research in the drug discovery process. New approaches like the combinatorial biosynthesis as well as combinatorial genomics have further strengthened the case of natural products for designing novel medicinal molecules.

Combinatorial biosynthesis essentially is genetic construction of new combination for screening the bioactives of the so formed hybrids. It essentially involves the genetic manipulation of genes in natural-product biosynthesis pathways as a way of producing natural-product analogs. Mutation or substitution of a gene in a biosynthetic pathway may result in synthesis of a previously unknown analog of a natural product (Hutchinson, 1998). Polyketides are small cyclized molecules. Around 5000 to 10,000 polyketides are known, and around 1% possess drug-like activity. A variety of polyketide drugs like Amphotericin B, Nystatin, Lovastatin, FK 506 are currently used by the pharmaceutical industry (Borchardt, 1999). The synthesis of polyketides is generally controlled by a set of enzymes referred to as Polyketide syntheses (PKS). Hopwood et al (1985) developed a method of producing hybrid antibiotics by recombining similar polyketide synthesis genes. However, minor modifications were obtained by his approach. Production of new polyketides is probably the most advanced application of combinatorial biosynthesis; genetically altering their biosynthesis pathways can potentially generate a nearly inexhaustible number of new polyketides for drug assays and further development. Khosla et al (1994) have developed a technique of chemobiosynthesis, which was used in altering PKS genes in order to make new polyketides. As many as 11 variants of Polyketides were generated by this approach; Kosan Biosciences at Hayward is currently involved in generating superior versions of erythromycin by chemobiosynthesis.

Oceanix Biosciences Corporation has recently developed a powerful set of biotechnology-based tool to enhance the discovery, development and production of new chemical entities for pharmaceutical industry by employing viable but non-cultivable microorganisms (VBNCs). It has been demonstrated that a majority of bacteria are viable but cannot be cultured under laboratory conditions. This implies that vast majorities of bioactives producers from the environmental samples have been missed in the drug discovery programs. Thus a protocol was designed to generate hybrid microorganisms that could express the environmental DNA of the VBNCs. This technology was termed as "combinatorial genomics". Combinatorial genomics is a direct, rapid and powerful set of manipulations that allow transfer and expression of random genetic material from the non-culturable to the donor or culturable (fermentative) microbes for the production of novel chemical structures.
or "unnatural" natural products (Maynak & Carlson, 1999). The combinatorial biosynthesis is focused on microbial pathways. There have also been advances in understanding of molecular biology behind the production of secondary metabolites from plants (Roessner & Scott, 1996; Dixon, 1999).

Thus modern pharmaceutical industry is using diverse approaches from traditional to those involving genomics for deriving the fascinating structures from the biodiversity in their drug discovery programs.

**The Future**

Natural products research is a very small part of the entire drug discovery process but it is the mainstay as it continues to provide a tremendous variety of lead structures, which serve as templates for the development of new drugs by the pharmaceutical industry. With advances in bioactive screening technology (high throughput assays) and in chemical methodology, natural products seem to be the best cost effective sources for new leads for tailoring therapeutic molecules. The sequencing of the human genome opens new territory in terms of our ability to identify the proteins expressed by genes associated with the onset of diseases. These proteins can be used as molecular targets for testing thousands of compounds, including natural products, in high throughput assays.

There is a huge potential for screening the biodiversity of plants and microbes using a variety of advanced techniques, as many of them have not been studied. It is also clear that as the purification, identification and testing technology improve, more and more natural products will become of interest to the pharmaceutical industry. Even rare and difficult samples may derive interesting compounds and with a variety of fishing techniques wherein the target macromolecules are bound to a matrix and then identified by mass spectral and NMR techniques.

**Conclusion**

There is little doubt that natural products will continue to have an important impact on the drug discovery process in pharmaceutical sector and it is equally imperative that we all work towards the preservation of the biodiversity and genetic diversity of the earth as a resource for future generations.

**References**

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