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Exploring the Potential of Natural Products in Cancer Treatment

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1.1 Introduction

Cancer remains a major cause of mortality worldwide. In 2006 in Europe there were an estimated 3.2 million cancer cases diagnosed (excluding non-melanoma skin cancers) and 1.7 million deaths from cancer (Ferlay *et al.*, 2007). According to the World Health Organization (www.who.int), from a total of 58 million deaths worldwide in 2005, cancer accounts for 7.6 million (or 13 %) of all deaths. Cancer rates are predicted to further increase if nothing changes, mainly due to steadily ageing populations in both developed and developing countries and current trends in smoking prevalence and the growing adoption of unhealthy lifestyles. It is estimated that almost half of cancer cases can be prevented by infection control, adoption of a healthy lifestyle (diet and exercise) and tobacco abstinence. Early diagnosis and effective treatment can further dramatically decrease cancer mortality. In the therapeutic area, the hope is to turn many cases of fatal cancer into 'manageable' chronic illness, as it has happened with other disease entities.

When it comes to treatment, humans have always turned to nature. The medicinal value of plants has been recognized by almost every society on this planet. Up to the nineteenth century, herbal extracts containing mixtures of natural products provided the main source of folk medicines. The first synthetic pharmaceutical drug, aspirin, was developed in the latter half of the nineteenth century and its potency as pain reliever spawned the era of synthetic therapeutic agents. Since the 1940s, 175 anticancer drugs have been developed and are commercially available in the United States, Europe and Japan; 65 % of these were inspired from natural products, i.e. pure natural products (14 % of total), semisynthetic modifications of

natural products, natural product mimics or synthetic molecules with pharmacophores from natural products (Newman and Cragg, 2007). The sheer numbers prove the importance and contribution of Nature's biodiversity to the development of efficient therapies.

At present, the role of natural products in drug discovery programmes of large pharmaceutical companies has been de-emphasized. The reasons for this situation can be traced back to the advent of high-throughput screening assays in the drug discovery process that favour single molecules and not mixtures of these (non-specific interferences, presence of fluorescent compounds and insoluble materials), the better suitability of combinatorial chemistry in this set-up and its 'overhyped' potential to deliver new lead compounds, the inherent difficulties of natural product research and the concerns about ownership. The Convention on Biological Diversity (www.cbd.int) in 1992 brought into the agenda the access of developing countries 'on a fair and equitable basis to the results and benefits arising from biotechnologies based upon genetic resources provided by these'. Therefore, apart from the risk of being called 'biopirates', pharmaceutical companies opted not to be engaged in natural product research, which suffers from lack of reproducibility of extracts, the inaccessibility of collection sites, laborious procedures to isolate and purify bioactive chemical compounds (often present in trace amounts), and the rediscovery of known compounds.

The impact of this shift has started to become obvious. Although molecular and cellular biology brought about a surge of molecular targets for therapy, this has not been accompanied by a rise in the number of anticancer drugs developed. Anticancer research has mainly focused on the cancer cells and the development of cytotoxic drugs for efficient and selective chemotherapy. Despite the identification of more than 100 distinct types of cancer and the puzzling findings of molecular and cellular biology, it has been suggested that there are six essential alterations in cell physiology that collectively dictate malignant growth: self-sufficiency in growth signals, insensitivity to growth-inhibitory signals, evasion of apoptosis, limitless replicative potential, sustained angiogenesis, and tissue invasion and metastasis (Hanahan and Weinber, 2000). Thus, the probability of finding one 'magic bullet' drug to cure cancer seems to be nil. Indeed, in chemotherapy, combinations of chemical compounds are used. In recent years, other non-cytotoxic therapeutic agents such as hormonal and biological response modalities are also under study. This is due to the realization that tumours are complex tissues in which mutant cancer cells have conscripted and subverted normal cell types to serve as active collaborators, and, furthermore, the realization of the potential of the immune system. This different approach (to restore balance) is common in many traditional holistic medicinal systems incorporating plants that boost the immune response, like *Withania somnifera* in ayurvedic medicine (Balachandran and Govindarajan, 2005).

The scope of this chapter is to illustrate the potential of natural products as sources of traditional and novel anticancer drugs, with emphasis on the philosophy and rationale directing source screening, lead compounds or new drug identification and subsequent drug development.

1.2 Sources

Plants and microorganisms have been the major sources of natural products throughout the centuries (Balunas and Kinghorn, 2005), but after one century of isolation and structural characterization of their natural products we realize that we have achieved only a glimpse into this vast reservoir of biodiversity. From the approximately 250 000–300 000 plants all over the world, about 10 % have been systematically investigated for the presence of bioactive

Trabectedin, 1

Figure 1.1 Ecteinascidin-743 (trabectedin or Yondelis) from *Ecteinascidia turbinata* is the first marine organism-derived anticancer drug. Photo Pharma Mar copyright

phytochemicals (McChesney *et al.*, 2007). However, these numbers do not reveal the wealth of natural products therein, since more than one bioactive natural product may be in one plant, either all through the plant or in a particular part (e.g. roots). Their concentration varies greatly according to developmental age, location, time, climate or environment; they may be produced only as a response to an elicitor (chemical communication, physical stimuli). Similarly, out of the million microorganisms, it is estimated that more than 99 % of these remain to be studied. The diversity of marine microorganisms is estimated to be more than 10 million species, more than 60 % of which are unknown (Jensen and Fenical, 1994). In addition to the open ocean, there are diverse and dynamic areas such as mangrove swamps, coral reefs, hydrothermal vents, and deep-sea sediments in which to search for microbes. Other marine organisms (e.g. sponges, tunicates) are an untapped source of novel natural products, which we have just started to explore. Marine organisms produce really novel and complex secondary metabolites. The richest sources of anticancer marine natural products have been soft-bodied and mainly sessile organisms, such as sponges, sea slugs and tunicates, which lack physical defence against their predators, and hence rely on chemical warfare using cytotoxic secondary metabolites. The first drug (ziconotide) isolated from marine organisms was launched in 2006 for chronic pain (Newman and Cragg, 2007), whereas several marine natural products are now tested for their anticancer efficacy in clinical trials (Simmons *et al.*, 2005). Yondelis (ecteinascidin-743, **1**) is a tris-tetrahydroisoquinoline isolated from the sea squirt, *Ecteinascidia turbinata* which received authorization in 2007 for commercialization from the European Commission for advanced soft tissue sarcoma (Figure 1.1). Ecteinascidin-743 is under study in two phase III clinical trials for the treatment of sarcoma and ovarian cancer and in other 16 phase I or phase II trials (www.clinicaltrials.gov). It interacts with the minor groove of DNA and alkylates guanine at the N2 position, which bends towards the major groove. Thus, the drug affects various transcription factors involved in cell proliferation, particularly *via* the transcription-coupled nucleotide excision repair system (von Mehren, 2007).

The list of potential sources of natural products can be extended to alcoholic and non-alcoholic beverages, processed food, animals, animal food and excreta, etc. (Tulp and Bohlin, 2004).

1.3 Different Approaches to the Search for Bioactive Natural Products

Given the wealth of sources, searching for a potent anticancer natural product does seem analogous to looking for a needle in a haystack. There are two main approaches to the study of those, the random screening and the rational selection. Random screening is nowadays possible, mainly in the pharmaceutical companies, due to the automated, high-throughput screening assays and the progress in isolation and fractionation techniques. Camptothecin (**2**), a potent cytotoxic alkaloid, was first extracted from the stem wood of the Chinese ornamental tree *Camptotheca acuminata* during the screening of thousands of plants in 1958 (Wall and Wani, 1995) (Figure 1.2). Monroe Wall and Mansukh Wani, while screening thousands of plant extracts as a possible source of steroidal precursors for cortisone, sent 1000 of the extracts to Jonathan Hartwell of the National Cancer Institute (NCI) Cancer Chemotherapy National Service Center (CCNSC) for investigation of their potential antitumour activity.

The extracts of *C. acuminata* were identified as the only ones showing significant activity in an adenocarcinoma assay. Camptothecin inhibits DNA topoisomerase I and the first

Camptothecin, **2**

Topotecan, **3**

Irinotecan, **4**

Figure 1.2 Camptothecin was originally isolated from *Camptotheca acuminata*. The analogues, topotecan and irinotecan are used in the clinical practice. Photos by Shu Suehiro

generation analogues, hycamtin (topotecan, **3**) and camptosar (irinotecan, CPT-11, **4**), marketed by Glaxo-SmithKline and Pfizer, respectively, are used for the treatment of ovarian and colon cancers (Figure 1.2). Over a dozen more camptothecin analogues are currently at various stages of clinical development (Sriram *et al.*, 2005; Srivastava *et al.*, 2005). The same research team discovered taxol (**5**) from the bark of the Pacific Yew tree (*Taxus brevifolia* Nutt.) as result of an exploratory plant screening programme sponsored by the USA National Cancer Institute (Figure 1.3). The discovery of taxol assumed an added measure of importance through the ground-breaking discovery by Horwitz and colleagues (Horwitz, 2004) of its unique mechanism of action, namely, 'its ability to induce the formation of characteristic microtubule bundles in cells'. In 1992, taxol was marketed for the treatment of refractory ovarian cancer and in 1994 for metastatic breast cancer. Taxotere (**6**), one of its semisynthetic derivatives was approved in 1996 for breast cancer and is now known as a better anticancer drug than taxol (Srivastava *et al.*, 2005) (Figure 1.3).

However, it is believed that the effectiveness of random screening in delivering new anti-cancer drugs or novel lead compounds is surpassed by that of the rational approach. With regards to plants, the most efficient approach has been the recording of the therapeutic uses of the plants in traditional or folkloric medicine systems. This field of research, ethnopharmacology, is part of ethnomedicine and ethnobotany. Information on the alleged medicinal uses of plants comes either from anthropological field studies or from study of ancient literature. There are many local communities in which healing traditions have only been transferred from one generation to the next orally and are now at a risk or are lost since those are displaced by modern western therapeutic systems. The fact that these have been refined through centuries

Figure 1.3 *Taxus brevifolia* was the original source of the anticancer drug taxol. The semisynthetic analogue taxotere is used for breast cancer. Photos by Walter Siegmund, retrieved from <http://en.wikipedia.org> on December 1 2007 and under GNU Free Documentation license

via trial and error and they have produced important anticancer drugs, such as podophyllotoxin, shows the necessity for taking actions for their preservation. The limited number of field ethnopharmacological studies at present can be attributed to the difficulties and the necessary expertise for the translation of indigenous diseases or concepts of illness into their modern counterparts and vice versa. Anticancer ethnopharmacological research is particularly problematic in that aspect, since cancer is not one disease entity and has multiple signs and symptoms. It has been suggested that the plants used for the following ethnomedical claims should be investigated; cancer treatment, treatment of 'hard swellings', abscesses, calluses, corns, warts, polyps, or tumours, immune disorders, infectious diseases, parasitic and viral diseases, abortive (Mans *et al.*, 2000; Hartwell, 1971). Another practice, though, is to give priority for anticancer research to plants with any alleged medicinal effect. The plants and their medicinal uses by indigenous healers must be recorded, specimens need to be taken back in the laboratories and be correctly identified by botanists, the herbal recipes, the dosage schedules, the total duration of therapy and the side effects must be observed and written down (Raza, 2006).

The species *Podophyllum peltatum* Linnaeus and *Podophyllum emodii* (Podophyllaceae) are the most representative example of plants with a long history of medicinal use for the treatment of cancer, i.e. treatment of skin cancers and warts (Figure 1.4). Two anticancer drugs, teniposide (**7**) and etoposide (**8**), which are semisynthetic derivatives of epipodophyllotoxin, show good clinical effects against several types of neoplasms including testicular and small-cell lung cancers, lymphoma, leukaemia, Kaposi's sarcoma, etc. (Figure 1.4). The major active constituent, podophyllotoxin, was first isolated in 1880, but its correct structure was only reported in the 1950s and it has not been used due to unacceptable toxicity. Podophyllotoxin inhibits the polymerization of tubulin and causes arrest of the cell cycle in the metaphase (Gordaliza *et al.*, 2004).

The development of well-organized electronic databases of relevant research work greatly helps to restrict the initial target group. In 2000 Graham *et al.* provided a list of over 350 plants that are used ethnomedically against cancer after a query in the NAPRALERT database. Apart from the useful input of ethnopharmacological research to modern drug discovery it also

Figure 1.4 Teniposide and etoposide are anticancer drugs, which are semisynthetic analogues of epipodophyllotoxin. Epipodophyllotoxin is a natural product present in the rhizome of *Podophyllum peltatum*. Photo by the United States Department of Agriculture, retrieved from <http://en.wikipedia.org> on December 1 2007 and in the public domain

offers improved therapeutic options in the regions of study through interaction of indigenous healers and physicians trained in western medicine. Furthermore, conservation and refinement of ethnomedicine will likely have a significant positive impact on global health, since more than 80 % of the population still rely on their traditional *materia medica* for their everyday health care needs.

Other rational approaches to the selection of plant or other sources for screening are the taxonomic, the phytochemical and that of chemical ecology. Screening within a given plant genus or family of particular interest (*taxonomic* approach), e.g. the genus *Taxus*, is a strategy which will likely reveal bioactive phytochemicals, especially of the same structural group, and thus promote the structure-activity studies. The high likelihood that a single compound may be present in several taxa of the same genus, and thus be responsible for the bioactivity of these, decreases the possibilities of finding a new lead compound. About 400 taxane-type diterpenoids have been isolated from various *Taxus* plants so far, and some of them possess interesting anticancer activities (Shi and Kiyota, 2005; Kingston and Newman, 2007). Furthermore, this screening introduced a solution to the problem of the supply of taxol. Taxol was isolated in a yield of 0.014 % from the extract of the bark of *Taxus brevifolia* in 1967; this low yield and the high quantities of taxol necessary drove *Taxus brevifolia* close to extinction. A biosynthetic precursor, 10-deacetylbaccatin III, was isolated from the needles of several yew trees, which are an abundant and renewable source, and served as a starting material for the semisynthesis of taxol or taxotere. Total synthesis of taxol, a great challenge to organic chemists, was reported in 1994 by Nicolaou *et al.* giving another solution to the production of taxol and other designed taxoids.

Focusing on a particular group of natural products, e.g. indole alkaloids, and screening all plants likely to contain those, constitutes the *phytochemical* approach, which is of particular significance to systematic botany. However, every group of natural products has a representative with anticancer activity. This has been traditionally performed *via* isolation and structural characterization of the natural products. The advances in molecular biology and genomics change the methodology in this traditional field; Zazopoulos *et al.* (2003) developed and used a high-throughput genome scanning method to detect and analyse gene clusters involved in natural-product biosynthesis. This method was applied to uncover biosynthetic pathways encoding enediyne antitumour antibiotics in a variety of actinomycetes and showed that a conserved cassette of five genes is widely dispersed among actinomycetes and that selective growth conditions can induce the expression of these loci, suggesting that the range of enediyne natural products may be much greater than previously thought. As discussed also in the taxonomic approach, it might be argued that screening in this way limits the possibility of finding novel lead compounds.

Microorganisms have produced in the past a significant number of FDA-approved anticancer drugs, such as bleomycin and doxorubicin, but present a particular problem as a source of natural products. The identity of most of them is not known and less than 1 % of these can be cultivated. A novel way of searching for natural products in these is the construction of environmental DNA libraries (Martinez *et al.*, 2000; Pettit, 2004). This method involves isolating large DNA fragments (100–300 kb) from microorganisms derived from soil, marine or other environments, and inserting these fragments into bacterial vectors, thus generating recombinant DNA libraries. Such libraries are then used to identify novel natural products by various means, including expression of the DNA in a heterologous host strain and screening for activities, or by directly analysing the DNA for genes of interest. Furthermore, the possibility of regulating the expression of isolated environmental gene

clusters or combining them with genes of other pathways to obtain new compounds (combinatorial biosynthesis) could be a further advantage over traditional natural product discovery methodologies.

Study of ecological interactions among organisms and between them and their environment, mediated by secondary metabolites that organisms produce (chemical ecology), e.g. plants poisonous to animals, has long been a successful strategy in discovering bioactive natural products (Harborne, 2001). One example of how this field changes under the light of findings from cellular and molecular biology is the search not for general cytotoxic compounds, but for effective nuclear factor (NF)- κ B inhibitors in marine organisms (Folmer *et al.*, 2007). Several bacteria and viruses have been reported to modulate NF- κ B activity in host cells in order to increase their chances to survive as parasites within the host. Pancer *et al.*, (1999) have shown that sea urchins use a NF- κ B analogue to protect themselves against apoptosis-inducing compounds released by the diatoms on which they graze, and to respond to bacterial infection and other pathogens. From the evolutionary point of view, one interesting potential explanation for the finding of NF- κ B inhibitors in marine organisms is the fact that marine invertebrates and fish, no matter how distantly related they appear to be, possess, in many cases, NF- κ B or closely related analogues (Folmer *et al.*, 2007).

1.4 Methodologies of Lead Compound or New Drug Identification

The tools for evaluating the 'anticancer' potential of natural products are rapidly increasing. Preclinical evaluation involves *in vitro* assays of the effect of natural products on specific molecular targets involved in apoptosis, mitosis, cell cycle control or signal transduction, *in vitro* evaluation of cytotoxicity or other mechanisms of action in cultured cancer cells and other normal cells, and evaluation of the antitumour activity in animal models (Mishra *et al.*, 2007). Bioactivity evaluation should also incorporate methods for evaluating the immunomodulating, anti-invasion or angiostatic potential of natural products. This vast array of available bioassays necessitates a strategic decision of which will be the first-line assays, which will determine the natural products that are candidates for the next round of bioactivity evaluation; it is obvious that the complete preclinical evaluation of all natural products is not possible not only because of incredibly high cost but also of ethical considerations. The recent overemphasis on the molecular targets is criticized as simplistic and reductionist, and the study of the effect at the cellular level is reappraised (Houghton *et al.*, 2007; Subramanian *et al.*, 2006). The case of the discovery of vincristine (9) and vinblastine (10), anticancer drugs approved in the early 1960s, which led to the semisynthesis of vinorelbine and vindesine, poses other interesting implications. *Catharanthus roseus* (former *Vinca rosea*) is used in ayurvedic medicine for the treatment of diabetes mellitus (Figure 1.5). In search for effective hypoglycaemic agents, Robert Noble and Charles Beer were surprised to observe that intravenous administration of the *C. roseus* extract to experimental mice resulted in a rapidly falling white blood count, granulocytopenia, and profoundly depressed bone marrow (Duffin, 2002). This chance observation led to the isolation and identification of vinblastine and vincristine as potent therapeutic agents and novel lead compounds.

Bioactivity evaluation is performed on isolated natural products and/or on extracts and/or purified fractions of those. The classic phytochemical approach has the risk of missing natural products that are in trace amounts, and, thus, rediscover known compounds,

Figure 1.5 *Catharanthus roseus* was traditionally used for treatment of diabetes but by chance discovery it was shown to possess anticancer activity. The useful anticancer agents, vincristine and vinblastine, were isolated from *C. roseus*. Photo by Conrado, retrieved from <http://en.wikipedia.org> on December 1 2007 and under GNU Free Documentation license

e.g. polyphenolics, in high abundance. Bioassay-guided fractionation is the most commonly used strategy for the identification of the bioactive lead compounds. Fractionation reduces complexity, increases the titer of low abundance components and removes 'nuisance' substances. The strategy followed for the isolation of camptothecin is shown in Figure 1.6.

Bioassay-guided fractionation can lead to 'strange' findings (Pieters and Vlietinck, 2005). The bioactivity of the extract might be higher than that of the isolated compounds or fractions and this may be attributed to synergy of the phytochemicals present or decomposition and/or oxidation of the phytochemicals due to the lower amounts of antioxidants present in the fractions and the materials/solvents used in the fractionation process. The possibility of the presence of not one active compound but of several is very strong. On the other hand, the bioactivity of the isolated compounds might be higher than 100 % of the extract due to competition of the phytochemicals present in the extract. The pharmacokinetics of the fractions may also be different from that of the extract (better or worse) since it has been shown that certain natural products affect absorption, e.g. tannins decrease absorption from intestine.

These procedures may lead to the re-isolation and re-identification of known compounds as the bioactive constituents, which is regarded as a considerable loss of time and funds in the search for novel bioactive lead compounds. Thus, de-replication is necessary at an early stage of the discovery process, preferably in the primary extracts, so as to allow the prioritization of work and concentration on those sources that produce novel compounds. Liquid chromatography–mass spectroscopy (LC-MS)/MS coupled with the on-line acquisition of UV/vis spectra and the construction of libraries is a tool for correct structure identification of phytochemicals in an extract (Fredenhagen *et al.*, 2005). Böröczky *et al.* (2006) suggested a simple gas chromatography-based method using cluster analysis as a data-mining tool to select samples of interest for further analysis of lipophilic extracts. Furthermore, the construction of natural product-focused spectral libraries of nuclear magnetic resonance data of isolated compounds allow for the rapid structural elucidation and thus an early de-replication (Dunkel *et al.*, 2006; López-Pérez *et al.*, 2007).

Once identified and the results of preclinical evaluation are good, the bioactive natural product will be directed to clinical evaluation. Results of clinical phase I and II trials will determine if the compound will be evaluated in phase III trials, will be sent back to the laboratory for optimization, or abandoned (Connors, 1996). On the way to the marketplace, the crucial problems of supply and large-scale production must be solved (McChesney *et al.*,

Figure 1.6 Bioassay-guided fractionation of *Camptotheca acuminata* wood plus wood bark. Bioactivity of the fractions was evaluated by the *in vivo* L1210 mouse life prolongation activity. Most of the chloroform phase after concentration was subjected to an 11-stage preparative Craig countercurrent distribution. The bioactive fractions were then combined and further purified by chromatography on a silica gel column and crystallization. The pure bioactive compound was proved to be camptothecin. Reproduced from Wall ME, Wani MC. Camptothecin and taxol: discovery to clinic – thirteenth Bruce F. Cain Memorial Award Lecture. *Cancer Res* 1995, **55**(4), 753–60, with permission from the American Association of Cancer Research

2007). Medicinal chemists play a key role in the generation of structural analogues and introduction of ‘drug-like’ features. Apart from the traditional chemoenzymatic approach, combinatorial chemistry of a natural product-lead compound is often involved in construction of libraries whereas combinatorial biosynthesis holds a great promise in the field (Boldi, 2004; Harvey, 2007). Combinatorial biosynthesis can be defined as the application of genetic engineering to modify biosynthetic pathways to natural products in order to produce new and altered structures using nature’s biosynthetic machinery (Floss, 2006; Julsing *et al.*, 2006). The introduced structural alterations range from simple reactions such as glycosylation, oxidations and reductions, methylations, isoprenylations, halogenations and acylations to the generation of complex hybrid ‘unnatural’ compounds. The screening of natural product libraries and extracts usually yields a substantially higher percentage of bioactive hits than that of

chemical libraries; a recent review (Berdy, 2005) estimates an approximately 100-fold higher hit rate for natural products.

1.5 Chemoprevention – A New Area for Natural Product Research

Epidemiological studies, showing that increased intake of fruits and vegetables is associated with reduced risk of cancer, triggered research on the identification and characterization of the biological properties of the natural products in edible plants and the creation of a new scientific field, that of chemoprevention (Reddy *et al.*, 2003). Chemoprevention, as a scientific field, may be considered still at its infancy, and includes the use of natural or pharmacological agents to suppress, arrest or reverse carcinogenesis, at its early stages. Studies, mainly *in vitro*, have shown that most dietary natural products exhibit pleiotropy; they affect several biological processes (even opposing functions) and act on a multitude of molecular targets (Reddy *et al.*, 2003; Russo, 2007). The ‘antioxidant’ effect is put forward by most scientists and helps unify the positive effects on different systems, e.g. cardiovascular, neurodegenerative diseases and cancer. Natural products, like genistein, resveratrol, curcumin, retinoic acid and epigallocatechin-3-gallate, became the focus of intense research and public interest. In parallel, a lot of dietary supplements, functional or medical foods, became available to the public and this created a lot of concerns about the safety, the quality, the efficacy and the legislative status of these products.

The field of the study of natural products as chemopreventive agents has to address many problems and challenges. A major problem is the confusion in the literature; from experiments in cell cultures with concentrations of natural products equal to or even higher than those appropriate for pharmacological agents and with no knowledge or study of the absorption and the bioavailability, some scientists jump to conclusions about anticancer or chemopreventive potential (Russo, 2007). Other important questions are ‘when’ the chemopreventive intervention must take place to show efficacy and what happens if the antioxidant treatment does not occur at the ‘appropriate time’ and especially what happens when it takes place with standard chemotherapy (Russo, 2007). The clinical evaluation of the chemopreventive properties of a natural product is particularly challenging due to the time involved, the lack of appropriate biomarkers and the fact that it involves healthy people. However, the fact that selective oestrogen receptor modulators, like tamoxifen and raloxifene, do decrease the incidence of breast cancer in post-menopausal women suggests a bright future for chemoprevention; raloxifene especially is a multifunctional medicine that was approved for reducing the risk of invasive breast cancer in postmenopausal women with osteoporosis and in postmenopausal women at high risk for invasive breast cancer (Jordan, 2007). Thus, it is possible that natural products, analogues or combinations of these will be used as chemopreventive agents. Furthermore, the public attention paid to dietary chemoprevention can only be viewed as positive since it increases the awareness of people of the significance of well-balanced diet rich in fruits and vegetables.

1.6 Concluding Remarks

Plants and microorganisms have been sources of a significant percentage of potent anticancer drugs used nowadays, although a small portion of these have been studied. Further exploration

of those, of marine organisms and of other novel sources will certainly reveal new drugs, novel lead compounds and new mechanisms of action. However, this process is time-consuming since it involves several steps: selection of the sources, screening and identification of bioactive drugs or lead compounds, *in vitro* and *in vivo* studies of the toxicity and mechanism of action, production or synthesis in high quantities, preclinical and clinical evaluation, approval and development of analogues with better characteristics which enter again the same cycle of drug development. Recognition that the biological diversity of the earth and, thus, the chemical diversity is rapidly diminishing is a very important stimulus for natural products research in the face of irreversible loss of sources of potential drugs. Moreover, in the light of increasing cancer rates, the area of cancer prevention using natural products is very important.

Progress can only be realized with sufficient funds. The immediate co-operation of universities, institutes, big pharmaceutical companies and small biotechnology firms is necessary in order to meet the demands for effective pharmaceuticals. Each sector can contribute in a different way; large-scale random high-throughput screening and clinical development can take place in pharmaceutical companies and in large institutes; universities and institutions can take on research directions that require lengthy procedures and are not so expensive, e.g. screening according to ethnopharmacology. The involvement of scientists from all fields in natural products research has and will further transform the field and the techniques involved in order to meet the demands of modern drug discovery and development.

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