Biodiversity as a Source of Anticancer Drugs

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Abstract: Natural Products have been the most significant source of drugs and drug leads in history. Their dominant role in cancer chemotherapeutics is clear with about 74% of anticancer compounds being either natural products, or natural product-derived. The biodiversity of the world provides a resource of unlimited structural diversity for bioprospecting by international drug discovery programs such as the ICBGs and NCDDGs, the latter focusing exclusively on anticancer compounds. However, many sources of natural products remain largely untapped. Technology is gradually overcoming the traditional difficulties encountered in natural products research by improving access to biodiverse resources, and ensuring the compatibility of samples with high throughput procedures. However, the acquisition of predictive biodiversity remains challenging. Plant and organism species may be selected on the basis of potentially useful phytochemical composition by consulting ethnopharmacological, chemosystematic, and ecological information. On the conservation/political front, the Convention on Biological Diversity (CBD) is allaying the anxiety surrounding the notion of biopiracy, which has defeated many attempts to discover and develop new natural products for human benefit. As it becomes increasingly evident and important, the CBD fosters cooperation and adaptation to new regulations and collaborative research agreements with source countries. Even as the past inadequacies of combinatorial chemistry are being analyzed, the intrinsic value of natural products as a source of drug leads is being increasingly appreciated. Their rich structural and stereochemical characteristics make them valuable as templates for exploring novel molecular diversity with the aim of synthesizing lead generation libraries with greater biological relevance. This will ensure an ample supply of starting materials for screening against the multitude of potentially "druggable" targets uncovered by genomics technologies. Far from being mutually exclusive, biodiversity and genomics should be the driving force of drug discovery in the 21st century.

Key Words: Natural products, biodiversity, drug discovery, anticancer, Convention on Biological Diversity, Bioprospecting, International Cooperative Biodiversity Groups, National Cooperative Drug Discovery Groups.

INTRODUCTION

Cancer is a major public health burden in developed countries. Global estimates revealed that there were 10.9 million new cases, 6.7 million deaths, and 24.6 million persons living with cancer in the year 2002 [1]. Cancer is the second leading cause of death in the United States [2], where one in four deaths is due to cancer. More than a million cases of cancer are diagnosed annually, and over 500,000 Americans die of cancer each year [3-4]. The four most common cancers, accounting for more than half of all cancer cases, are those of the prostate, breast, lung, and colon/rectum.

The etiology of major cancers remains largely unknown. Current dogma suggests a clear distinction between cancer chemoprevention and chemotherapy. While chemotherapy manages established disease, traditionally, cancer chemoprevention has been defined as an intervention in carcinogenesis, facilitated by blocking induction of the neoplastic process ("blocking agents"), or preventing transformed cells from progressing to the malignant phenotype ("suppressing agents") [5]. Chemoprevention may also involve the reversal of progression.

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Even with the availability of a large number of cancer drugs and cancer treatment options, the need persists for more efficacious and less toxic cancer chemotherapeutic agents. Food-based compounds (e.g., genistein) targeted to signaling pathways and crucial molecular targets also hold promise of clinical efficacy against cancer in a chemopreventive setting. The world's biodiversity has proven to be an important source of notable anticancer and chemopreventive agents to date [6-10]. We believe it will represent one of the most important resources and options in the search for new anticancer drugs well into the future.

BIODIVERSITY AS A RESOURCE FOR NEW DRUG DEVELOPMENT

Biodiversity is defined as the variability among living organisms from all sources including, among others, terrestrial, marine and other aquatic ecosystems, and the inseparable ecological complexes with which they interact [11-14]. It also includes diversity within species, between species and of ecosystems [14]. While the total number of organism species on earth is not known, the total number of species that have been described is known to be in the range of 1,392,485 [15] to 1,750,000 [16]. A modern version of *Systema Naturae* [17] perpetuates the legacy of Linnaeus in the 21st century.

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A 5-kingdom comprehensive classification system of earth's biodiversity [18] in current use is made available online as part of the *Systema Naturae* [17]. An alternative six-kingdom classification received additional support recently [19]. In the case of taxonomic categories below kingdom or phylum, one or more specific taxonomic classification systems have been published for certain organism groups, such as the flowering plants [20-24]. The most comprehensive links for the study of the biodiversity are provided by the National Center for Biotechnology Information [25].

Presently, the six-kingdom classification (Table **1**) has become the standard in many works [26]. Clearly, the 1,085,000 species of arthropods, which include insects, make up the greatest portion (75.4%) of the earth's biodiversity. Insects alone comprise 62% of global biodiversity. Despite the fact that the number of insect species is estimated to be in the range of 2,000,000 - 100,000,000 [26], this group remains relatively untouched as a source of novel compounds by the drug discovery community. Animal species [Kingdom Animalia], excluding the arthropods comprise 13%, while the fungi and the protists [protoctists] represent 4% and 5% of global biodiversity, respectively. It is reasonable to assume that the overwhelming majority of fungal species have yet to be isolated and tested for the production of biologically-active compounds, even though members of the order Actinomycetales, like Streptomycetes, have been the most prominently known microbial producers of natural products.

Based on the number of known species (300,000-500,000) [26], plants represent the second largest source of biodiversity (15%). Despite their popularity in traditional and alternative health systems of the world, and their indispensable role in the primary health care of populations in developing countries, only 15 – 20 % of terrestrial plants have been evaluated for their therapeutic potential. Advances in sample selection and collection methodologies, chemical separation technologies and the superior sensitivity of modern mechanism-based assays will likely pave the way for discoveries beyond the basic structural types and biosynthetic pathways that have already been described in plants.

Current perception holds that bacteria and fungi will be the most probable sources of new natural products because of their long evolutionary history, their varied metabolisms, and their use of small molecules to sense and modulate their environment. The number of sequenced bacterial and fungal genomes is also rapidly increasing. It is known that 99.9% of microbial genetic diversity cannot be cultured with traditional methods. However, in principle, the biologically active small molecules made by the uncultured microbial majority could be accessed directly through isolation of environmental DNA and heterologous expression in a cultured host. This genome-driven strategy, coupled with other innovative technology platforms comprising genomics, microbiology and natural product chemistry will allow greater access to these elusive sources of secondary metabolites in the future.

Marine ecosystems dominate the planet and host biodiversity at least equal to, and by some estimates far in excess of that found on land. Although the marine environment may contain over 80% of the world's plant and animal species [28], it remains largely unknown and untapped as a source of new biologically active molecular motifs with improved scope for drug discovery. Marine natural products chemistry is also a relatively young science, with chemical ecology likely to take center stage as the field matures. Even so, it is clear at this juncture that extraordinary chemical diversity is the hallmark of marine natural products structures; these compounds often possess exotic functional groups (e.g. isonitrile, multiple halogenation) or unique parent carbon skeleta that are without precedent among their terrestrial counterparts. Increasing sophistication in the tools available for deep-sea exploration will expand the habitats that can be sampled, thus, improving the opportunities for discovery of novel active metabolites from marine sources. The escalating number of marine-derived compounds undergoing various

Table 1. Partial Classification of Living Organisms[a]

1)	**Kingdom EUBACTERIA** (bacteria, cyanobacteria "blue-green algae", etc.) [c. 4000 described species]
2)	**Kingdom ARCHAEA** (halobacterians, methanogens, eocytes, etc.)
3)	**Kingdom [EUKARYA] PROTOCTISTA** (protozoa, "algae", etc.) [80,000 described species]: Actinopoda [6,000]; Foraminifera [10,000]; Ciliophora [8,000]; Sporozoa [5,000]; Rhodophyta (red algae) [5,000]; Gamophyta (green algae) [10,000]; Bacillariophyta (diatoms) [12,000]
4)	**Kingdom [EUKARYA] PLANTAE** (land plants) [270,000 described species]: Mosses and liverworts [16,000]; Pteridophytes (ferns) [10,000]; Spermatophyta (seed plants) [240,000]
5)	**Kingdom [EUKARYA] FUNGI** (molds, lichen-forming, yeasts, mushrooms, etc.) [72,000]
6)	**Kingdom [EUKARYA] ANIMALIA** [1,320,000]: Mesozoa (small, ciliated, wormlike, marine parasitic invertebrates)[b]; Metazoa [1,320,000]; Porifera (sponges) [10,000]; Cnidaria (hydras, jellyfish, corals, etc.) [10,000]; Platyhelminths (flatworms) [20,000]; Nematoda (roundworms) [25,000]; Echinodermata (sea urchins, etc.) [6,000]; Chordata (fish, birds, mammals, etc.) [45,000]; Arthropoda (crabs, spiders, insects, etc.) [1,085,000] [of which, 950,000 belong to the insects]; Mollusca (snails, squids, etc.) [70,000]; Annelida (segmented worms) [12,000]
Viruses [c. 4,000]	

[a] Only groups equivalent to Phylum level and above with more than 5,000 described species are included in the list [26].

[b] Mesozoa comprises 38 species [27].

stages of clinical development attests to the growing recognition of marine biodiversity as a source of anticancer agents.

BIODIVERSITY AS A SOURCE OF ANTICANCER AGENTS

Despite the colossal pool of available organism species, the number of biodiversity-derived anticancer drugs in clinical use is very small. It was revealed in a landmark survey in 2003 [29], that 61% of 877 small molecule new chemical entities (NCEs) introduced worldwide from 1981 to 2002 could be traced to natural products. Considering only anticancer drugs in the same time frame, 48 of the 65 (74%) small molecule NCEs either were natural products, were based thereon, or mimicked them in one form or another. In addition, considering the 126 small molecule anticancer drugs effectively available in the West and Japan between 1940s and 2002, 62% were nonsynthetic in origin. Table **2** highlights the most notable of these clinically established anticancer compounds from the perspective of their source organisms. Table **3** supplements this essential list with anticancer agents that were derived through synthetic modification.

EFFORTS IN THE SEARCH FOR NEW ANTICANCER DRUGS FROM THE WORLD'S BIODIVERSITY

The largest effort in the search for new anticancer drugs from the world's biodiversity was launched by the National Cancer Institute (NCI), in 1955 [32]. Between 1960 and 1982, about 114,000 extracts from an estimated 35,000 plant samples (representing 12,000-13,000 species) collected mostly from temperate regions of the world had been screened against a number of tumor systems (primarily, L1210 and P388 mouse leukemias) [32, 33]. The program was extended from 1986 to 2004, with an emphasis on global plant collections and screening against tumor cell cultures. A wide variety of compound classes were isolated and characterized. Clinically significant chemotherapeutic agents that emerged from this program included paclitaxel (*Taxus brevifolia* Nutt. and other *Taxus* species), hycamptamine (topotecan), CPT-11, and 9-aminocamptothecin. The latter three compounds are semisynthetic derivatives of camptothecin (*Camptotheca acuminata* Decne.) [34].

As an extension of the NCI's effort in the search for anticancer agents, the NCDDG (National Cooperative Drug Discovery Group) program was established in 1983 by the Developmental Therapeutics Program, Division of Cancer Treatment and Diagnosis [35, 36]. This program supports broad, innovative, and multi-disciplinary approaches to the discovery of new, synthetic or natural-source derived anticancer agents. Toward the end of April, 2005, only two NCDDGs in operation focus their effort in the search for anticancer agents from natural sources [35, 36]. Accomplishments of the groups have recently been reviewed [37-41]. As a result of the overall NCDDG effort, three anticancer agents, topotecan (1996), carmustine/Gliadel (1996), and DAB389IL-2 (1998) were discovered and marketed following approval by the United States Food and Drug Administration (FDA), while eight other drugs reached IND status [42]. The development of another promising compound, cryptophycin, was halted due to untoward toxicities encountered during Phase II clinical trials for the treatment of solid tumors [35]. Cryptophycin, a novel depsipeptide isolated from *Nostoc* sp. (Nostocaceae, Cyanobacteria), destabilizes microtubules and induces Bcl-2 phosphorylation leading to apoptosis [43].

Table 2. Partial List of Biodiversity-Derived Drugs Available Commercially for the Treatment of Cancer.[a]

Drug Generic Name (year introduced)	Source
Aclarubicin (1981)	*Streptomyces galilaeus* Ettlinger [Actinomycetales, Kingdom Eubacteria]
Arglabin (1999)	*Artemisia glabella* Kar. & Kir. [Asteraceae, Spermatophyta, Kingdom Plantae]
Doxorubicin (1974)	*Streptomyces peucetius* [Actinomycetales, Kingdom Eubacteria]
Masoprocol (1992)	*Larrea tridentata* (Sessé & Moc. ex DC.) Coville [Zygophyllaceae, Spermatophyta, Kingdom Plantae]
Mitomycin C (1974)	*Streptomyces caespitosus* [Actinomycetales, Kingdom Eubacteria]
Paclitaxel (1992)	*Taxus brevifolia* Nutt. and *Taxus* spp. (Taxaceae, Spermatophyta, Kingdom Plantae)
Pentostatin (1992)	*Streptomyces antibioticus* (Waksman & Wodruff) Waksman & Henrici [Actinomycetales, Kingdom Eubacteria]
Peplomycin (1981)	*Streptomyces verticillus* Takita [Actinomycetales, Kingdom Eubacteria]
Solamargine (1987)	*Solanum incanum* L. [Solanaceae, Spermatophyta, Kingdom Plantae]
Vinblastine (1965)	*Catharanthus roseus* (L.) G.Don [Apocynaceae, Spermatophyta, Kingdom Plantae]
Vincristine (1963)	*Catharanthus roseus* (L.) G. Don [Apocynaceae, Spermatophyta Kingdom Plantae]

[a] Sources: The United States Food and Drug Administration [30], The European Agency for the Evaluation of Medicinal Products (EMEA) [31], and various other sources.

Table 3. Partial list of Biodiversity-Derived Semisynthetic Drugs Available Commercially for Cancer Chemotherapy[a]

Drug Generic Name (year introduced)	Parent Natural Product and Source Organism
Amrubicin hydrochloride (2002)	Anthracycline-derived [*Streptomyces* spp., Kingdom Eubacteria]
Docetaxel (1995)	Taxol-derived [*Taxus brevifolia*, Spermatophyta, Kingdom Plantae]
Elliptinium acetate (1983)	Synthetic modification from ellipticine [*Bleekeria vitiensis* A.C. Sm., Apocynaceae, Kingdom Plantae]
Epirubicin hydrochloride (1984)	Anthracycline-derived [*Streptomyces* spp., Kingdom Eubacteria]
Etoposide phosphate (1996)	Podophyllotoxin-derived [*Podophyllum peltatum* L., Podophyllaceae, Kingdom Plantae]
Idarubicin hydrochloride (1990)	Anthracycline-derived [*Streptomyces* spp., Kingdom Eubacteria]
Irinotecan hydrochloride (1994)	Camptothecin-derived [*Camptotheca acuminata* Decne., Nyssaceae, Kingdom Plantae]
Peplomycin (1983 Japan)	Bleomycin-derived [*Streptomyces verticillus* Takita, Kingdom Eubacteria]
Pirarubicin (1998)	Anthracycline-derived [*Streptomyces* spp., Kingdom Eubacteria]
Teniposide (1992)	Podophyllotoxin-derived [*Podophyllum peltatum* L., Kingdom Plantae]
Topotecan hydrochloride (1996)	Camptothecin-derived [*Camptotheca acuminata* Decne., Kingdom Plantae]
Valrubicin (1999)	Anthracycline-derived [*Streptomyces* spp., Kingdom Eubacteria]
Vindesine (1980 Europe; 1985 Japan)	Vinca alkaloid-derived [*Catharanthus roseus*, Kingdom Plantae]
Vinorelbine (1989)	Vinca alkaloid-derived [*Catharanthus roseus*, Kingdom Plantae]
Zinostatin stimalamer (1994)	A conjugate of poly(styrene-comaleic acid) and neocarzinostatin [*Streptomyces* sp., Kingdom Eubacteria]

[a]Sources: The United States Food and Drug Administration [30] and The European Agency for the Evaluation of Medicinal Products (EMEA) [31], and various other sources.

Other outstanding developments include: bengamide A and psammaplin A (and related compounds), from marine sponges *Jaspis* sp. (Jaspidae, Porifera) and *Druinella purpurea* (Druinellidae, Porifera), respectively [37]; various cytotoxic compounds from marine heterotrophic bacteria, dinoflagellates (microalgae) and fungi [38]; a series of Pol β lyase and DNA polymerase β inhibitors from plants [39]; MDR-selective agent austocystin D from *Aspergillus* sp. (Trichocomaceae, Ascomycota); cytotoxic namenamicin from *Polysyncraton lithostrotum* (Didemnidae, Ascidiacea), a Metazoan ascidian; the tubulin inhibitor, hemiasterlin (HT-286) from *Cymbastela* sp. (Gadidae, Demospongiae), a marine sponge collected in Papua New Guinea [40]; pervilleine A [44-45], the tropane alkaloid P-glycoprotein inhibitor from *Erythroxylum pervillei* Baill. (Erythroxylaceae, Spermatophyta), and cytotoxic rocaglates silvestrol and episilvestrol from *Aglaia foveolata* Pannell (Meliaceae, Spermatophyta) [46].

Commencing in 1993, the International Cooperative Biodiversity Groups (ICBG), a program administered by the Fogarty International Center (FIC), National Institutes of Health (NIH), and supported through funds from NIH, National Science Foundation (NSF) and US Department of Agriculture (USDA) Foreign Agricultural Service (FAS), started operation in an effort to integrate the following goals: improvement of human health through drug discovery, incentives for conservation of biodiversity, and development of new models of sustainable economic activity that focus on the environment, health, equity and democracy. The implementation of this program is based on the belief that the discovery and development of pharmaceutical and other useful agents from the world's biodiversity can, under appropriate circumstances, promote scientific capacity development and economic incentives to conserve the biological resources from which these products are derived [47]. The drug discovery effort is focused on a broad range of target organisms, comprising five (Eubacteria, Protoctista, Plantae, Fungi, Animalia) of the six kingdoms of overall biodiversity [48].

The University of Illinois at Chicago (UIC)-based ICBG, entitled "Studies on Biodiversity of Vietnam and Laos", serves as a model for the implementation of the ICBG principles, which are, ultimately, the principles of the United Nations Convention on Biological Diversity [14, 49-53]. In its current Phase II operation (2003-2008), this ICBG consortium consists of two US-based academic institutions (UIC and Purdue University), two Vietnamese research institutions (Vietnamese Academy of Science and Technology, and Cuc Phuong National Park), one Lao research institution (Traditional Medicine Research Center), and an industrial partner (Bristol-Myers Squibb, BMS).

The drug discovery and development goal of the UIC-ICBG is to uncover biologically active molecules from plants of Vietnam and Laos as chemotherapeutic candidates for malaria, tuberculosis, AIDS and cancer. Approaches

utilized in the selection of plants consist of biodiversity-based collection ["random" collection] centered in the Cuc Phuong National Park in Vietnam, and ethnobotany-driven interviews on the medicinal uses of plants in Laos.

CONVENTION ON BIOLOGICAL DIVERSITY AND INTELLECTUAL PROPERTY ISSUES

From our direct participation in the NCI [54, 55], NCDDG [41], and ICBG [49-52] efforts in the worldwide search for anticancer agents from terrestrial plants, we can say with conviction that such an endeavor is a complex process that demands the involvement of not only scientific expertise, but also expertise in a broad spectrum of human endeavors including diplomacy, international laws and legal understandings, social sciences, politics, anthropology, and basic common sense. Equally important is the fact that such endeavors must be governed by international bureaucratic and regulatory procedures. As recently as 1983, the world's biodiversity was considered to be the heritage of mankind, which might be used without restriction [56]. Because of the historical inequity in the use of the biodiversity, and the benefits that resulted from such use, between the owners of the biodiversity and the users [57], the idea of a common heritage became untenable. This led to the drafting and eventual signing of the United Nations Convention on Biological Diversity (CBD) [14] in 1992, which sets as its objectives "the conservation of biological diversity, the sustainable use of its components and the fair and equitable sharing of the benefits arising out of the utilization of genetic resources" (Article 1 of the CBD). These principles, and the fact that the Convention recognizes the sovereign right of each state over its genetic resources, make international cooperation fundamental in any project exploring global biodiversity as source materials for pharmaceutical discovery, an endeavor known widely as "bioprospecting".

Central to any bioprospecting effort is an international agreement signed by all parties, or a set of coordinated inter-institutional agreements, that cover access to genetic resources (biodiversity); intellectual property (IP) resulting from the cooperation; sharing of short- and long-term benefits arising from discovery and commercialization of new drugs; and conservation of the biological resources for future generations. If an ethnobotanical or ethnomedical approach is used in the bioprospecting effort, additional specific terms relating to prior informed consent (PIC) and recognition of Indigenous Intellectual Property Rights (IIPR) must be incorporated into the agreement. Benefit-sharing is a critical point in negotiating agreements: improving international scientific collaboration and models for long-term distribution of royalties are typical subjects for discussion. The Letter of Collection used by the NCI to provide an umbrella agreement to its plant contractors in implementing their exploration and collection programs [35] is a pioneering example of such international arrangements.

In all the biodiversity-based NCDDG projects, the issues of access to genetic resources, recognition of IP and IIP, PIC, and benefit-sharing are routinely handled on a case-by-case basis. These issues assume greater importance in the ICBG program, since the latter is an experiment by the US government in the implementation of the CBD. A number of model agreements have been created as a result of compliance with the project standards [58]. An analysis of the UIC-based ICBG agreement has been published and serves as a model to illustrate the complexity of an ICBG Memorandum of Agreement (MOA) [53].

THE SEARCH FOR PREDICTIVE BIODIVERSITY

A number of items must be considered in planning and implementing a bioprospecting effort. The approach to selecting the organisms to be investigated has been the subject of discussion and debate. Four primary approaches are usually adopted: a) the ethnobotanical approach; b) the biodiversity-driven approach, often referred to as the “random collection” approach; c) the chemotaxonomic approach; and d) the ecological approach, based on field observations of the interactions between organisms [55]. An ethnobotanical approach is often, but not always, used for terrestrial plants, because of the close interactions between plants and people and the use of plants in day-to-day health care in indigenous communities. In the case of marine habitats or microbial samples, the biodiversity-based approach has been typical. Even so it may be stressed that most marine secondary metabolites possess intrinsic biological relevance for certain human diseases (e.g., cancer and infectious diseases), since these compounds evolved for defense and competition for space. Furthermore, there is evidence that some natural products isolated from marine invertebrates may actually be produced by associated microorganisms [59], making fermentation an integral part of the sample acquisition process. Microbial fermentation or organism culture is also an important method for producing biomass to provide adequate sample amounts for larger-scale analyses [60]. In the shift toward more rational and predictive approaches to bioprospecting, it is anticipated that chemical ecology will play a greater role in guiding the pursuit for active molecules from plants, and other terrestrial and marine invertebrates. The understanding of biotic interactions/communications at the molecular level may hold the key to uncovering natural products of greater pharmacological relevance. The Panama-ICBG capitalized on ecological criteria based on plant-insect interactions [61]. Another obvious rationale-directed approach that might also offer cost-effectiveness is recourse to the knowledge base and heritage available in traditional systems of medicine of various countries. An attempt could be made to determine commonality in uses for similar disease conditions across diverse human cultures prior to the initiation of phytochemical work.

Collection methodology that facilitates and promotes accurate recollection is essential to ensure a reliable supply of biomass for larger-scale compound isolation, typically to meet the requirements of *in vivo* and late-stage preclinical studies. This includes good field documentation, use of Global Positioning System (GPS) to pinpoint site locations, mapping of sites and the ready availability of superior computer database support. The field data for the UIC ICBG program are posted on the Internet in the Atlas of Seed Plants of Cuc Phuong National Park (http://uic-icbg.pharm.uic.edu) as an example. This information is also available in hardcopy form [62]. Good collection methodology is also important for marine sites, though collection technology is different [63]. Needless to say, precise taxonomic identifica-

tion of organisms involved either in the field, or with subsequent support from taxonomic specialists, is crucial for accurate recollection of organisms or targeted collection of specific organisms [55].

Plant tissue or cell culture technologies are suited for scale-up production of bioactive metabolites once they have been determined to be of interest [64, 65]. Plant cell culture has also been applied to eliminate the uncertainty of re-accessing native plant samples exhibiting interesting chemistry. Further development of sustainable supplies of compounds for clinical trials or commercialized drugs is critical, and may proceed by exploring sustainable harvest methods, cultivation (including aquaculture), microbial fermentation, genetic engineering, and semi-synthesis or synthesis of candidate drugs or analogs [66]. This will ensure adequate supply of the compound while protecting the source organism and its habitat from overexploitation. Planning for sustainability should begin early in product development [34, 67].

THE UNIVERSITY OF ILLINOIS AT CHICAGO – ICBG

Integrated Drug Discovery Platform in a Highly Collaborative Environment

Plant collections, taxonomic identifications, and recollections are performed on a regular and continuing basis. Libraries of plant extracts are assembled for future re-examination in new biological systems. New compounds that are routinely isolated based on a particular biological activity contribute to our pure natural products libraries, which may in turn be exposed to multiple screens across our ICBG program.

Drug discovery in an academic setting follows the classic activities of sample collection, biological screening and phytochemical investigation. The involvement of diverse disciplines in-house ensures the optimization of the technology platform surrounding our natural products drug discovery effort. Processing time of crude extracts is reduced by automation whenever possible. Both methanolic and $CHCl_3$- or CH_2Cl_2-soluble extractives are assayed in our target-based high-throughput systems based on the necessity for eliminating tannins and polyphenols, which have been found to interfere with certain enzyme-based assays. Clinically-relevant and sensitive bioassays are employed for efficient detection of hidden chemical diversity, and serve to guide the iterative deconvolution of chemically complex plant extracts.

Plant materials having confirmed activity in our high throughput systems are analyzed using an effective combination of expertise and advanced separation technology. Our fractionation/isolation protocol is typically based on the combination of flash (Isco CombiFlash SG100C Separation System), semi-preparative and preparative HPLC chromatography. Droplet counter-current systems are also employed to maximize efficiency. Traditional methods employed include gravity, vacuum, flash, or low-medium pressure column chromatography using a variety of adsorbents and media. Bioassay-directed fractionation and isolation is coupled with early LC/MS/MS-based dereplication strategies that favor novel bioactive molecules over nonspecific, known and/or unwanted compounds from mixtures. Chemical profiling by HPLC-MS is especially relevant for chemotaxonomically-related species under investigation.

Structures of active compounds are elucidated by state-of-the-art physical and spectroscopic methods such as high resolution electron impact mass spectrometry (HREIMS), chemical ionization (CI), field desorption and fast-atom bombardment (FAB) MS. Stereochemistry is determined by use of ORD and CD coupled with high-field 1H- and 13C-NMR spectroscopy, with appropriate two-dimensional (2D) and decoupling experiments. Single crystal X-ray crystallographic analysis is also routinely carried out to solve difficult stereochemistry assignments.

The modern drug discovery environment demands rapid screening, hit identification and hit-to-lead development. Advancements in analytical technology and automation have increased the competitiveness of academic laboratories. In many cases, however, industry support is critical for academic innovation. Companies are poised to provide the support/services traditional natural products research establishments need to bridge the gap between technological innovation, and traditional knowledge and experience, which undoubtly still remain indispensable. ICBG lead compounds that meet BMS' criteria for advanced preclinical development will be supported through the pipeline.

In addition to pharmacokinetics, oral bioavailability, toxicology, pharmaceutics, and synthesis of lead compounds which match the preclinical lead profile criteria, other lead optimization strategies may include combinatorial chemistry. Nature's biodiversity represents a valuable reservoir of small molecules that may serve as novel scaffolds for the construction of a variety of lead generation libraries with anticancer attributes. In addition, by mimicking the *in vivo* distribution properties of natural compounds, a substantially more diverse set of combinatorial products may be produced that could also have greater biological relevance.

Lead compounds will also be considered as candidates for more advanced testing in mechanism-based screens covering a broad range of molecular targets that have been implicated in the control of cancer cell growth and metastasis. These may involve the modulation of processes involved in angiogenesis, cell cycle control, signal transduction and hormonal pathways.

Overall Accomplishments

For the period of 1998-2005, 3,331 plant samples have been collected from CPNP, comprising about 950+ species of flowering plants identified to species level. Since the results of our biotic inventory indicate that 1,926 species of Angiosperms (Spermatophyta) [62] occur at Cuc Phuong National Park, 900+ species remain to be examined. In addition, 960 plant samples (about 700+ species) have been collected based on ethnobotanical field interviews.

Of these 3,331 samples, extracts of 2,309 (comprising at least 800 species) have been assayed in our infectious diseases assays and tumor cell line panel. This has resulted in 22 plant recollections and the isolation of ~280 pure natural products of varying degrees of structural complexity and/or biological activity from plant leads identified on the basis of

their anti-HIV, anti-malaria, anti-tuberculosis or anticancer activities. Aside from the discovery of biologically active compounds, our studies have contributed significantly to the knowledge of natural products chemistry with 80 new secondary metabolites being reported for the first time from higher plants. The large percentage (*ca.* 29%) of our isolates that are novel chemical entities may well be the result of our rigorous prioritization criteria based primarily on preexisting biological and chemical information on both the plant genus and species in question. Furthermore, 47 of these 80 novel compounds possess varying degrees of biological activity. This informatics-based dereplication strategy is effectively coupled with resources and expertise available in an academic setting to achieve a dynamic level of productivity in the delivery of novel and active compounds. The chemical diversity of these new natural products includes alkaloids/amides, macrocyclics, lignans, neolignans, butenolides, phenylpropanoids, sesquiterpenes, norditerpenes, triterpenes and steroids [68-71].

Of the 80 new chemical entities, 10 belong to a novel carbon skeleton being described for the very first time. These structurally-related anti-HIV sesquiterpenes (litseaverticillols A - J) were isolated from *Litsea verticillata* Hance (Lauraceae). These compounds are unique in that that they are α, β-conjugated pentacyclosesquiterpenes with a 9-member sidechain [72-74].

Identification of Plant-derived Chemotherapeutic and Chemopreventive Agents

Since the implementation of our ICBG anticancer drug discovery program 5 years ago, 65 anticancer compounds have been obtained, 30 of which are novel. These compounds are in various stages of preclinical testing including evaluation in animal models.

During the present cycle of our ICBG program, cytotoxicity screening is being performed with 3 carcinoma cell lines, Lu-1 (lung), LNCaP (prostate), MCF-7 (breast) and HUVECs (human umbilical vein endothelial cells) using the sulforhodamine B (SRB) protein staining methodology [75, 76]. These cell lines represent tumors of greatest significance to mankind. HUVECs were also employed as a test system for identifying samples with potential antiangiogenic activity. In addition, our vast experience with general cytotoxicity screens involving thousands of extracts and a broad panel of cell lines has not revealed selectivity towards any particular type of tumor cell. Pure compounds are considered active if their CC_{50} values are less than 4 μg/ml. This threshold for activity was derived from our cumulative experience in anticancer drug discovery from higher plants. This level of stringency results in a consistent hit rate of about 10%.

Our plant samples are also being evaluated in luciferase reporter gene assays based on the activity of a panel of gene promoters with well-established connections to the carcinogenic process, for example nuclear factor kappa B (NFκB) [77]. In addition, reporter cell lines based on other promoters have been constructed. These include promoters for antioxidant response element (ARE), activating protein-1 (AP-1), estrogen response element (ERE), hypoxia inducible factor-1 response element (HRE), mdm2 (p53 response element), peroxisome proliferator-activated receptors (PPAR), and xenobiotic response element (XRE).

It has been pointed out in various perspectives and review articles [78] that there is not a well-defined boundary between the chemotherapeutic and chemopreventive activity of most potential drug leads. Chemoprevention assays that are being conducted involve induction of phase II drug metabolizing enzymes such as quinone reductase (QR) [79], and inhibition of enzymes involved in estrogen (aromatase) [80, 81], and prostaglandin (COX-2) biosynthesis [82-84].

Work is generally performed on promising lead compounds to elucidate their mechanism(s) of action, and to provide an initial indication of clinical potential with relevant *in vivo* models. Potential chemotherapeutic compounds that are active in the murine hollow fiber assay [85, 86] are further tested in standard xenograft models to verify their *in vivo* anticancer activity. Methodologies have been established for multiple myeloma [87, 88], melanoma [89], and breast tumors [90, 91]. A panel of mouse or rat models for human cancer is also in place to test the anticarcinogenic efficacy of newly discovered chemopreventive compounds. These models evaluate the chemopreventive effect of agents on mammary gland, lung, prostate, bladder, colon and skin carcinogenesis under the influence of various pharmacokinetic variables. *In vivo* components are emphasized since no interest in development will be generated prior to demonstration of activity in animal models. *In vivo* tests require larger amounts of compound, and these acquisitions will be based on the structures of the leads. Possibilities include large-scale re-isolation, synthesis or semi-synthesis. In these cases, sufficient interest should be generated to solicit the assistance of the NCI [RAID (Rapid Access to Intervention Development) program] or our industrial partner.

BIODIVERSITY-BASED THERAPEUTIC STRATEGIES OF THE FUTURE

The generalized loss of growth control exhibited by cancer cells is the net result of accumulated abnormalities in multiple cell regulatory systems such as cell signaling, regulation of cell cycle, and the control of programmed cell death. Recent insights into the molecular mechanisms underlying neoplastic disease, and the plethora of molecular targets emanating from the global genome sequencing effort have presented new approaches for disrupting tumor-specific cell signaling, cell survival, mitosis, gene expression, macromolecular synthesis and angiogenesis. The empirical strategy directing cancer chemotherapeutic discovery has been replaced by a new paradigm that demands a more reasoned and knowledge-based approach. A new generation of anticancer agents is being developed based on the extension of cancer research to a more specific understanding of the molecular mechanisms of neoplasia.

Targeted therapy refers to development of drugs targeted at a protein or mechanism of action that is biologically and clinically relevant to cancer itself. As a result of this selective molecular approach, targeted therapies are expected to be more efficacious and less toxic to non-cancerous cells than currently available agents that inhibit cellular proliferation. The translation of novel molecular targets into useful

approaches for clinical development has resulted in a number of FDA-approved agents such as imatinib/Gleevecä, gefitinib/Iressa®, erlotinib/Tarceva®, cetuximab/Erbitux®, bortezomib/Velcade®, fulvestrant/Faslodex®, anastrozole/ Arimidex® , and bevacizumab/Avastin®, to name a few. Experience with conventional cytotoxic therapy and with experimental agents suggests that combining these new, targeted drugs with either conventional therapies or one another is likely to provide the best efficacy. Some of the most attractive targets for molecular-targeted cancer chemotherapeutic strategies include the family of receptor and non-receptor tyrosine kinases (RTKs); growth factors and growth factor receptors [e.g., epidermal growth factor receptor, (EGFR) and type 1 insulin-like growth factor receptor (IGFR)]; the Ras/Raf/MAP kinase pathway (e.g., ras farnesylation); the phosphatidylinositol-3 kinase (PI3K)/Akt/ PTEN pathway; c-Src signaling; mammalian target of rapamycin (mTOR); antimitotics [e.g., cyclins and cyclin-dependent kinases (CDKs)]; modulators of apoptosis [e.g., caspases, poly(ADP-ribose) polymerase-3 (PARP-3), and Bcl-2/BclXL]; telomerase; histone deacetylase (HDAC); hypoxia-inducible factor-1α (HIF-1α); heat shock protein 90 (hsp90); nuclear factor kappa B (NFκB); ubiquitin-mediated protein degradation pathway; retinoid X receptors [RXRs]; peroxisome proliferator-activated receptors (PPARs); prostaglandin synthetic pathways [cyclooxygenase-2 (COX-2) and lipoxygenase (LOX)]; steroid hormone receptors [e.g., estrogen receptor, (ERβ)]; vitamin D receptor and angiogenesis-associated factors [e.g., vascular endothelial cell growth factor (VEGF)] [92-94]. Natural product inhibitors of a select group of novel molecules that are being investigated as viable targets for chemotherapeutic intervention will be reviewed elsewhere in this issue. Although numerous signal transduction modulating drugs and mechanistically-based strategies are on the horizon or already in early clinical trials, only time will reveal those that are most clinically relevant and/or superior.

A complementary new view to the adoption of more rational therapeutics for cancer is based on the "systems approach" [95], in which delineation of aberrant/dysfunctional signal transduction systems manifested in different tumor cell types might allow eventual prediction of tumor types most amenable to therapy. Although signal transduction modulation in cancer therapy has reached a state of maturity and many elements of these critical signaling pathways constitute validated targets, some of these approaches have not been successful clinically because of the redundancies and overlapping nature of complex signal transduction pathways. Several critical signaling nodes will have to be disrupted in order to attain optimal outcomes given the complex crosstalk between signaling pathways. Therefore, the selection of inhibitors with a range of inhibitory potencies might prove to be valuable [96]. By implication, the use of overly selective kinase antagonists may be problematic in achieving useful activity in solid tumors.

Various molecular targets of chemoprevention are also relevant to the therapy of cancer. Regardless of whether a chemopreventive or chemotherapeutic approach is considered, it is clear that cancer is a multifactorial disease that requires modulation of multiple pathways and multiple targets. This less specialized function fits the pharmacological profile of many nutrients and phytochemicals such as genistein [97-109] and other soy isoflavones [110-113], green tea polyphenols [114-121], and (-)-epigallocatechin-3-gallate (EGCG) [122-127], silibinin [128, 129], lycopene [130-136], curcumin [137-143], perillyl alcohol [144-150], resveratrol [151-157] and grape seed proanthocyanidins [158-159]. The key molecular targets of many of these natural products are being elucidated in the midst of an increasing appreciation for their often pleiotropic molecular mechanisms of action [160-163]. These compounds might reasonably be incorporated into various regimens as single agents to prevent the occurrence or recurrence of cancer, or in combination with chemotherapy to treat cancer [96]. Phytochemicals such as lycopene, genistein and soy isoflavones, perillyl alcohol, grape seed proanthocyanidins and green tea polyphenols are presently being tested in NCI-sponsored clinical trials for the chemoprevention and chemotherapy of prostate and breast cancers. Indeed, the selection of these micronutrients for testing in clinical trials is based on the totality of evidence arising from epidemiologic, *in vitro*, animal, and clinical studies [164]. Research priorities for the future include an investigation into the possible molecular targets for micronutrients, and genetic and epigenetic factors that dictate direction and magnitude of a clinical response. Phytochemicals and micronutrients are, therefore, poised to expand the potential contributions of biodiversity to cancer control.

DISCUSSION

Traditionally, the earth's biodiveristy has been a major source of new drugs, and many successful drugs were originally synthesized to mimic the action of natural product molecules [165]. Although natural products have consistently provided high quality leads for the development of new therapeutics, the investment in natural products research has declined substantially during the last decade, during which combinatorial chemistry became the major source of new chemical entities in drug discovery [166]. However, despite the increased speed of synthesis, combinatorial approaches have yet to generate any real increase in the number of lead optimization candidates or drugs [167].

In addition to their great structural diversity, natural products are highly evolved to perform a function by interacting with specific biomolecules in the organism. This has raised the proposition that essentially all natural products possess some receptor binding capacity [168]. Molecules from nature are relevant to all biological processes even though they did not evolve to interact with human proteins specifically. Additionally, since there are elements of conservatism in all proteins (e.g., in the structural components of protein domains), natural products should harbor intrinsic utility as a source of drug leads. This biological relevance would also imply that natural products are suitable probes for chemogenomics. The contributions of proteomics, structural biology and genomics have yielded an overwhelming number of potential molecular targets that will undoubtedly require the types of physical, chemical and spatial characteristics well furnished by compounds from nature. To that end, there is renewed interest in natural product scaffolds as templates for the synthesis of novel molecular diversity.

Indeed, a statistical investigation into the structural characteristics of natural products revealed that compounds from nature represent a structurally unique and innovative pool of potential lead structures that cannot simply be replaced by synthetic efforts (such as that provided by combinatorial approaches) [169, 170]. In general, natural products have higher molecular weights than synthetic compounds due to the incorporation of fewer nitrogen, halogen, or sulfur atoms. Molecular rigidity and the high number of chiral centers also ensure selectivity, given the stereospecificity of most biological targets.

State-of-the-art developments in combinatorial chemistry, high throughput screening, bioinformatics and genomics are being integrated widely into the field of drug discovery. The challenges once faced by multidisciplinary groups in accessing the diverse chemical space provided by natural products efficiently and effectively are also being surmounted by new technologies. Hit validation and hit-to-lead development can be expedited by optimizing the technology platform surrounding the discovery process. Fundamentally, natural products research can now be interfaced with the speed and specifications of many target-based high throughput screens. In order to meet the demands of the genomics age, the chemical investigation of samples from biodiverse sources may ultimately submit to a new paradigm. Instead of bioactivity-guided fractionation, a more effective approach may be to separate crude samples into as many chemically interesting compounds/fractions as possible as a basis of forming/assembling libraries of isolated or concentrated natural products for future screening purposes. Sensitive and state-of-the-art structure elucidation methods may now be integrated into the high throughput platform.

The discipline of natural products research may be on the verge of undergoing a renaissance. While keen interest will remain in the use of gene transfer technologies at various stages of the drug discovery process, it should be noted that at present, however, an incomplete knowledge of many metabolic pathways limits work at the molecular level. Meanwhile, chemogenomics [171] is an emerging drug discovery discipline that has garnered much widespread enthusiasm and support. With the help of robust information technology systems, large chemical libraries are judiciously screened for the parallel identification of potential therapeutic targets together with their ligands or modulators of activity. Cancer research is particularly poised to take advantage of the rational and expedited nature of chemogenomics, as the approach is able to identify and validate personalized treatment strategies that will selectively target the most prevalent and/or significant genetic alterations that form the basis of a particular neoplasm.

As in the case with the ultimate clinical application of targeted and personalized therapies for cancer, the promise of chemogenomics has yet to be realized. Fundamental to the success of this endeavor is the judicious selection and examination of diversity-enriched chemical libraries specifically to provide ligand probes for the multitude of genetic sequences uncovered by the global genome sequencing effort. The stagnating diversities of existing chemical libraries have presumably deterred efforts to understand the pathophysiological roles of many of these putative molecular targets, and to assess their relevance for therapeutic intervention. It is envisioned that natural products contained in the world's biodiversity will bridge this gap by virtue of the magnitude and complexity of their structural diversity, and their intrinsic property of being evolutionarily optimized within biological systems as a whole.

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ABBREVIATIONS

ICBG	=	International Cooperative Biodiversity Group
NCDDG	=	National Cooperative Drug Discovery Group
NCI	=	National Cancer Institute
FDA	=	Food and Drug Administration
CBD	=	Convention on Biological Diversity
FIC	=	Fogarty International Center
NIH	=	National Institutes of Health
NSF	=	National Science Foundation
USDA	=	US Department of Agriculture
FAS	=	Foreign Agricultural Service
UIC	=	University of Illinois at Chicago
BMS	=	Bristol-Myers Squibb
IP	=	Intellectual property
PIC	=	Prior informed consent
IIPR	=	Indigenous Intellectual Property Rights
MOA	=	Memorandum of Agreement
KB	=	Oral epidermoid carcinoma
Col-2	=	Colon carcinoma
LNCaP	=	Prostate carcinoma
Lu-1	=	Lung carcinoma
MCF-7	=	Breast carcinoma

HL-60 = Promyelocytic leukemia
HUVECs = Human umbilical vein endothelial cells
NFκB = Nuclear factor kappa B
ARE = Antioxidant response element
AP-1 = Activating protein-1
ERE = Estrogen response element
HRE = Hypoxia inducible factor-1 response element
mdm2 = p53 response element
PPAR = Peroxisome proliferator-activated receptors
XRE = Xenobiotic response element
QR = Quinone reductase
COX-2 = Cyclooxygenase -2
RAID = Rapid Access to Intervention Development
RTKs = Receptor tyrosine kinases
EGFR = Epidermal growth factor receptor
IGFR = Type 1 insulin-like growth factor receptor
mTOR = Mammalian target of rapamycin
CDKs = Cyclin-dependent kinases
PARP-3 = Poly(ADP-ribose) polymerase-3
HDAC = Histone deacetylase
HIF-1α = Hypoxia-inducible factor-1α
hsp90 = Heat shock protein 90
RXRs = Retinoid X receptors
LOX = Lipoxygenase
ERβ = Estrogen receptor beta
VEGF = Vascular endothelial growth factor

REFERENCES

[1] Parkin, D.M.; Bray, F.; Ferlay, J. and Pisani, P. (2005) *CA Cancer J. Clin.*, **55**, 74-108.
[2] Hoyert, D.L.; Kung, H.C. and Smith, B.L. (2005) *Natl. Vital. Stat. Rep*., **53**(15), 1-48.
[3] Jemal, A.; Tiwari, R.C.; Murray, T.; Ghafoor, A.; Samuels, A.; Ward, E.; Feuer, E.J. and Thun, M.J. (2004) *CA Cancer J. Clin.*, **54**, 8-29.
[4] American Cancer Society Facts and Figures (2005). American Cancer Society, Atlanta, p. 1.
[5] Sporn, M.B. and Hong, K.W. (1997) *Science*, **278**, 1073-1077.
[6] Kinghorn, A.D.; Farnsworth, N.R.; Beecher, C.W.W.; Cordell, G.A.; Pezzuto, J.M.; Wall, M.E.; Wani, M.C.; Brown, D.M.; O'Neill, M.J.; Lewis, J.A. and Besterman, J.M. (1995) *Int. J. Pharmacog*., **33** (Suppl.), 48-58.
[7] Kinghorn, A.D.; Su, B.N.; Jang, D.S.; Chang, L.C.; Lee, D.; Gu, J.Q.; Carcache-Blanco, E.J.; Pawlus A.D.; Lee, S.K.; Park, E.J.; Cuendet, M.; Gills, J.J.; Bhat, K.; Park, H.S.; Mata-Greenwood, E.; Song, L.L.; Jang, M. and Pezzuto J.M. (2004) *Planta Med*., **70**(8), 691-705.
[8] Powis, G. Ed. (1994) *Anticancer Drugs: Antimetabolite Metabolism and Natural Anticancer Agents*, International Encyclopedia of Pharmacology and Therapeutics, Section 140, Pergamon Press, Oxford, UK.
[9] Yun, T.K. (1999) *Ann. N.Y. Acad. Sci*., **889**, 157-192.
[10] Park, E.J. and Pezzuto, J.M. (2001) in *Encyclopedia of Pharmaceutical Technology*, Edn. 2., (Swarbrick, J. and Boylan, J.C., Eds.), Marcel Dekker, New York, pp. 97-113.
[11] U.S. Congress Office of Technology Assessment (1987) Technologies to Maintain Biological Diversity, OTA-F-330, US Government Printing Office, Washington, DC., ch.1, p. 3. Available at http://www.wws.princeton.edu/cgi-bin/byteserv.prl/~ota/disk2/1987/8727/8727.PDF
[12] WRI, IUCN and UNEP (1992) in *Global Biodiversity Strategy: Guidelines for Action to Save, Study and Use Earth's Biotic Wealth Sustainably and Equitably*. WRI, Washington, DC; IUCN, Gland, Switzerland; UNEP, New York, 260 pp. Available at http://ceres.ca.gov/ceres/calweb/biodiversity/def_WRI.html
[13] UNEP World Conservation Monitoring Centre (2003) Strategic Plan for the Convention on Biological Diversity. Available at http://www.unep-wcmc.org/gbc/cbd.htm
[14] Secretariat of the Convention on Biological Diversity (2001) *Handbook of the Convention on Biological Diversity*, Earthscan Publications, Ltd., London and Sterling, VA, pp. 3-26. Available at http://www.biodiv.org/convention/articles.asp
[15] Wilson, E.O. (1988) in *Biodiversity - The current state of biological diversity*, (Wilson, E.O. and Peter, F.M, Eds.), National Academy Press, Washington, D.C., pp. 3-18.
[16] Hammond, P.M. (1995) in *Global Biodiversity Assessment*, Chapter 3.1, (Heywood, V.H. and Watson, R.T., Eds.), Cambridge University Press, Cambridge, p. 113-138.
[17] Brands, S.J. (comp.) (1989-2005) in *Systema Naturae 2000*, Amsterdam, The Netherlands. Available on line at http://www.taxonomicon.net/ and http://sn2000.taxonomy.nl/Taxonomicon/ProjectDescription.aspx
[18] Margulis, L. and Schwartz, K.V. (1998) *Five Kingdoms. An Illustrated Guide to the Phyla of Life on Earth*, W.H. Freeman, San Francisco. Available at http://sn2000.taxonomy.nl/Taxonomicon/TaxonTree.aspx?id=1&groupingBy=0.1
[19] Cavalier-Smith, T. (2004) *Proc. Roy. Soc. Lond.*, **271**, 1251-1262.
[20] Thorne, R.F. (1976) *A phylogenetic classification of angiosperms in Evolutionary Biology*, (Hecht, M.C., Steere, W.C. and Wallace, B., Eds.), vol. **9**, Plenum Press, N.Y., pp. 35-106.
[21] Dahlgren, R.M.T. (1975) *Bot. Notis.*, **128**, 119-147.
[22] Dahlgren, G. (1989) *Bot. J. Linn. Soc.*, **100**, 197-203.
[23] Takhtajan, A.L. (1980) *Bot. Rev.*, **46**, 225-359.
[24] Cronquist, A. and Takhtajan, A.L. (1993) *An Integrated System of Classification of Flowering Plants*, 2nd ed., Columbia University Press, New York.
[25] NCBI (National Center for Biotechnology Information) (2005) *Taxonomy Resources*. Available at http://www.ncbi.nlm.nih.gov/Taxonomy/taxonomyhome.html/index.cgi?chapter=resources [NCBI homepage at http://www.ncbi.nlm.nih.gov/]
[26] Hammond, P.M. (1995) Chapter 3.1, in *Global Biodiversity Assessment*, (Heywood, V.H. and Watson, R.T., Eds.), Cambridge University Press, Cambridge.
[27] European Register of Marine Species (2005). Available at http://erms.biol.soton.ac.uk/lists/full/Mesozoa.shtml
[28] Ocean 98 Foundation; affiliated with UNESCO; http://www.ocean98.org and Ocean Voice International (http://www.ovi.ca)
[29] Newman, D.M.; Cragg, G.M. and Snader, K.M. (2003) *J. Nat. Prod.*, **66**(7), 1022-1037.
[30] http://www.fda.gov/cder/cancer/approved.htm and http://www.fda.gov/cder/ob/default.htm
[31] http://www.emea.eu.int/htms/human/epar/a-zepar.htm
[32] Cragg, G.M. and Boyd, M. (1996) in *Medicinal Plant Resources of the Tropical Forest*, (Balick, M.J., Elisabetsky, E. and Laird, S.A., Eds.), Columbia University Press, New York, pp. 101-136.
[33] Cragg, G.M.; Simon, J.E.; Jato, J.G. and Snader K.M. (1996) in *Progress in New Crops*, (Janick, J., Ed.), ASHS Press, Arlington, VA, pp. 554-560.
[34] Cragg, G.M.; Schepartz, S.A.; Suffness, M. and Grever, M.R. (1993) *J. Nat. Prod*., **56**(10), 1657-1668.
[35] Hallock, Y.F. and Cragg, G.M. (2003) *Pharm. Biol*., **41** (Suppl.), 78-91.
[36] NCI-DTP-GCOB (2005) National Cooperative Drug Discovery Groups. Available at http://dtp.nci.nih.gov/branches/gcob/gcob_web3.html.
[37] Crews, P.; Gerwick, W.H.; Schmitz, F.J.; France, D.; Bair, K.W.; Wright, A.E. and Hallock, Y. (2003) *Pharm. Biol*., **41** (Suppl.), 39-52.
[38] Fenical, W.; Jensen, P.R.; Kauffman, C.; Mayhead, S.L.; Faulkner, D.J.; Sincich, C.; Rao, M.R.; Kantorowski, E.J.; West, L.M.; Strangman, W.K.; Shimizu, Y.; Li, B.; Thammana, S.; Drainville,

K.; Davies-Coleman, Kramer, R.A.; Fairchild, C.R.; Rose, W.C.; Wild, R.C.; Vite, G.D. and Peterson, R.W. (2003) *Pharm. Biol*., **41** (Suppl.), 6-14.
[39] Hecht, S.M. (2003) *Pharm. Biol.*, **41** (Suppl.), 68-77.
[40] Ireland, C.M.; Aalbersberg, W.; Andersen, R.J.; Ayral-Kaloustian, S.; Berlinck, R.G.S.; Bernan, V.; Carter, G.; Churchill, A.C.L.; Clardy, J.; Concepcion, G.P.; Dilip de Silva, E.; Discafani, C.; Fojo, T.; Frost, P.; Gibson, D.; Greenberger, L.M.; Greenstein, M.; Harper, M.K.; Mallon, R.; Loganzo, F.; Nunes, M.; Poruchynsky, M.S. and Zask, A. (2003) *Pharm. Biol*., **41** (Suppl.), 15-38.
[41] Kinghorn, A.D.; Farnsworth, N.R.; Soejarto, D.D.; Cordell, G.A.; Swanson, S.M.; Pezzuto, J.M.; Wani, M.C.; Wall, M.E.; Oberlies, N.H.; Kroll, D.J.; Kramer, R.A.; Rose, W.C.; Vite, G.D.; Fairchild, C.R.; Peterson, R.W. and Wild, R. (2003) *Pharm. Biol*., **41** (Suppl.), 53-67.
[42] NCI-DTP website at http://dtp.nci.nih.gov/branches/gcob/gcob_ web3.html.
[43] Smith, C.D.; Zhang, X.; Mooberry, S.L.; Patterson, G.M. and Moore, R.E. (1994) *Cancer Res.* **54**, 3779-3784.
[44] Mi, Q.; Cui, B.; Silva, G.L.; Lantvit, D.; Lim, E.; Chai, H.; You, M.; Hollingshead, M.G.; Mayo, J.G.; Kinghorn, A.D. and Pezzuto, J.M. (2001) *Cancer Res.*, **61**(10), 4030-4037.
[45] Silva, G.L.; Cui, B.; Chavez, D.; You, M.; Chai, H.B.; Rasoanaivo, P.; Lynn, S.M.; O'Neill, M.J.; Lewis, J.A.; Besterman, J.M.; Monks, A.; Farnsworth, N.R.; Cordell, G.A.; Pezzuto, J.M. and Kinghorn, A.D. (2001) *J. Nat. Prod.*, **64**(12), 1514-1520.
[46] Hwang, B.Y.; Su, B.N.; Chai, H.; Mi, Q.; Kardono, L.B.; Afriastini, J.J.; Riswan, S.; Santarsiero, B.D.; Mesecar, A.D.; Wild, R.; Fairchild, C.R.; Vite, G.D.; Rose, W.C.; Farnsworth, N.R.; Cordell, G.A.; Pezzuto, J.M.; Swanson, S.M. and Kinghorn, A.D. (2004) *J. Org. Chem*., **69**(10), 3350-3358. Erratum in: *J. Org. Chem.*, 2004 **69**(18), 6156.
[47] Fogarty International Center (2004) International Cooperative Biodiversity Groups. Available at http://www.fic.nih.gov/ programs/ icbg.html
[48] NIH News 2003. Third Round Awards are Announced Under Interagency Biodiversity Program. Available at http://www.nih.gov/ news/pr/dec2003/fic-16.htm
[49] Soejarto, D.D.; Gyllenhaal, C.; Regalado, J.C.; Pezzuto, J.M.; Fong, H.H.S.; Tan, G.T.; Hiep, N.T.; Xuan, L.T.; Binh, D.Q.; Hung, N.V.; Bich, T.Q.; Thin, N.N.; Loc, P.K.; Vu, B.M.; Southavong, B.H.; Sydara, K.; Bouamanivong, S.; O'Neill, M.J.; Lewis, J.; Xie, X.-M. and Dietzman, G. (1999) *Pharm. Biol*., **37** (Suppl.), 100-113.
[50] Soejarto, D.D.; Gyllenhaal, C.; Regalado, J.C.; Pezzuto, J.M.; Fong, H.H.S.; Tan, G.T.; Hiep, N.T.; Xuan, L.T.; Hung, N.V.; Bich, T.Q.; Loc, P.K.; Vu, B.M.; Southavong, B.H.; Sydara, K.; Bouamanivong, S.; O'Neill, M.J. and Dietzman, G. (2002). *Nat. Prod. Sci*., **8**, 1-15.
[51] Soejarto, D.D.; Xuan, L.T.; Vu, B.M.; Dac, L.X.; Bich, T.Q.; Southavong, B.H.; Sydara, K.; Bouamanivong, S.; Zhang, H.J.; Fong, H.H.S.; Tan, G.;.Pezzuto, J.; Franzblau, S.G.; Gyllenhaal, C.; Riley, M.C.; Hiep, N.T.; Loc, P.K. and Hung, N.V. (2002) in *Intellectual Property Rights and Traditional Knowledge on Genetic Resources in Pharmaceutical and Cosmetic Business, JBA/NITE, International Symposium* 2002, 14 November, 2002, Tokyo, Japan, pp. 47-83.
[52] Soejarto, D.D.; Gyllenhaal, C., Tarzian Sorensen, J.A.; Fong, H.H.S.; Xuan, L.T.; Binh, L.T.; Hiep, N.T.; Hung, N.V.; Vu, B.M.; Bich, T.Q.; Southavong, B.H.; Sydara, K. and Pezzuto, JM. (2003) in *Proceedings of The 2nd International Conference on Medicinal Mushroom and the International Conference on Biodiversity and Bioactive Compounds (BioThailand 2003)*, 17-19 July, Peach, Pattaya, Thailand, BIOTEC, NSTDA, and Ministry of Science and Technology, Thailand, pp. 5-22.
[53] Soejarto, D.D.; Gyllenhaal, C.; Fong, H.H.S.; Xuan, L.T.; Hiep, N.T.; Hung, N.V.; Bich, T.Q.; Southavong, S.; Sydara, K. and Pezzuto, J.M. (2004) *J. Nat. Prod.*, **67** (Spec. Issue), 294-299.
[54] Soejarto, D.D. (1993) in *Human Medicinal Agents from Plants*, (Kinghorn, A.D. and Balandrin, M., Eds.), American Chemical Society, Washington, DC., pp. 98-111.
[55] Soejarto, D.D. (1996) *J. Ethnopharmacol*., **51**, 1-5.
[56] FAO (1983) Annex to Resolution 8/83. International Undertaking on Plant Genetic Resources. Twenty-second Session of the FAO Conference, Rome 1983. Available at ftp://ext-ftp.fao.org/ag/cgrfa/ Res/C8-83E.pdf
[57] Ten Kate, K. and Laird, S.A. (1999) in *The Commercial Use of Biodiversity*. Earthscan Publications, London, pp. 1-12.
[58] Rosenthal, J.P. (1996) in *Proceedings of the OECD International Conference on Biodiversity Incentive Measures*, Cairns, Australia.
[59] Haefner, B. (2003) *Drug Discov. Today*, **8**(12), 536-544.
[60] Munro, M.H.; Blunt, J.W.; Dumdei, E.J.; Hickford, S.J.; Lill, R.E.; Li, S.; Battershill, C.N. and Duckworth, A.R. (1999) *J. Biotechnol*., **70**(1-3), 15-25.
[61] Coley, P.D.; Heller, M.V.; Aizprua, R.; Araúz, B.; Flores, N.; Correa, M.; Gupta, M., Solis, P.N.; Ortega-Barría, E.; Romero, L.I.; Gómez, B.; Ramos, M.; Cubilla-Rios, L.; Capson, T.L. and Kursar, T.A. (2003) *Front. Ecol. Env*., **1**(8), 421–428.
[62] Soejarto, D.D.; Hiep, N.T.; Loc, P.K.; Cuong, N.M.; Bien, L.K.; Dai, T.D. and Kadushin, M.R. (2004) in *Seed Plants of Cuc Phuong National Park, Vietnam. A Documented Checklist*, Cuc Phuong National Park, Ninh Binh, Vietnam. Agricultural Publishing House, Hanoi, Vietnam, pp. i-xxxiv, 1-760, plates I-XCIX.
[63] Wright, A.D.; Konig, G.M.; Angerhofer, C.K.; Greenidge, P. and Linden, A. (1996) *J. Nat. Prod*., **59**(7), 710-6.
[64] Kirakosyan, A.; Sirvent, T.M.; Gibson, D.M. and Kaufman, P.B. (2004) *Biotechnol. Appl. Biochem.*, **39**(Pt 1), 71-81.
[65] DiCosmo, F. and Misawa, M. (1995) *Biotechnol. Adv.*, **13**(3), 425-53.
[66] Wender, P.A.; Hinkle, K.W.; Koehler, M.F. and Lippa, B. (1999) *Med. Res. Rev*., **19**(5), 388-407.
[67] Cragg, G.M. (1998) *Med. Res. Rev*., **18**(5), 315-331.
[68] Zhang, H.-J.; Tamez, P.A.; Hoang, V.D.; Tan, G.T.; Hung, N.V.; Xuan, L.T.; Cuong, N.M.; Thao, D.T.; Soejarto, D.D.; Fong, H.H.S. and Pezzuto, J.M. (2001) *J. Nat. Prod*., **64**(6), 772-777.
[69] Zhang, H.-J.; Qiu, S-H.; Tamez, P.; Tan, G.T.; Aydogmus, Z.; Hung, N.V.; Cuong, N.M.; Angerhofer, C.; Soejarto, D.D.; Pezzuto, J.M. and Fong, H.H.S. (2002) *Pharm. Biol*., **40**(3), 221-224.
[70] Zhang, H.-J.; Tamez, P.; Hashimoto, K.; Nakata, M.; Aydogmus, Z.; Tan, G.T.; Hung, N.V.; Xuan, L.T.; Cuong, N.M.; Soejarto, D.D.; Pezzuto, J.M. and Fong, H.H.S. (2002) *Planta Med*., **68**(12), 1088-1091.
[71] Chien, N.Q.; Hung, N.V.; Santarsiero, B.D.; Mesecar, A.D.; Cuong, N.M.; Soejarto, D.D.; Pezzuto, J.M.; Fong, H.H. and Tan, G.T. (2004) *J. Nat. Prod*., **67**(6), 994-998.
[72] Hoang, V.D.; Tan, G.T.; Zhang, H.-J.; Tamez, P.A.; Hung, N.V.; Cuong, N.M.; Soejarto, D.D.; Fong, H.H.S. and Pezzuto, J.M. (2002) *Phytochemistry* **59**, 325-329.
[73] Zhang, H.-J.; Tan, G.T.; Hoang, V.D.; Hung, N.V.; Cuong, N.M.; Soejarto, D.D.; Pezzuto, J.M. and Fong, H.H.S. (2003) *Tetrahedron* **59**(2), 141-148.
[74] Zhang, H.-J.; Hung, N.V.; Cuong, N.M.; Soejarto, D.D.; Pezzuto, J.M.; Fong, H.H.S. and Tan, G.T. (2005) *Planta Med.*, **71**(5), 452-457.
[75] Skehan, P.; Storeng, R.; Scudiero, D.; Monks, A.; McMahon, J.; Vistica, D.; Warren, J.T.; Bokesch, H.; Kenney, S. and Boyd, M.R. (1990) *J. Natl. Cancer Inst*., **82**, 1107-1112.
[76] Rubinstein, L.V.; Shoemaker, R.H.; Paull, K.D.; Simon, R.M.; Tosini, S.; Skehan, P.; Scudiero, D.A.; Monks, A. and Boyd, M.R. (1990) *J. Natl. Cancer Inst*., **82**, 1113-1118.
[77] Baldwin, A.S. (2001) *J. Clin. Invest.*, **107,** 241-246.
[78] Mehta, R.G and Pezzuto, J.M. (2002) *Curr. Oncol. Rep.*, **4**, 478-486.
[79] Kang, Y.-H. and Pezzuto, J.M. (2004) *Methods Enzymol*., **382**, 380-414.
[80] Stresser, D.M.; Turner, S.D.; McNamara, J.; Stocker, P.; Miller, V.P.; Crespi, C.L. and Patten, C.J. (2000) *Anal. Biochem.*, **284**, 427-430.
[81] Bhat, K.P. and Pezzuto, J.M. (2001) *Arch. Pharm. Res*., **24**(6), 473-484.
[82] Rimando, A.; Cuendet, M.; Desmarchelier, C.; Mehta, R.G.; Pezzuto, J.M. and Duke, S.O. (2002) *J. Agric. Food Chem.*, **50**, 3453-3457.
[83] Cuendet, M. and Pezzuto, J.M. (2000) *Drug Metabol. Drug Interact.*, **17**(1-4), 109-157.
[84] Su, B.N.; Jones, W.P.; Cuendet, M.; Kardono, L.B.; Ismail, R.; Riswan, S.; Fong, H.H.; Farnsworth, N.R.; Pezzuto, J.M. and Kinghorn, A.D. (2004) *Phytochemistry*, **65**(21), 2861-2866.
[85] Hollingshead, M.G.; Alley, M.C.; Camalier, R.F.; Abbott, B.J.; Mayo, J.G.; Malspeis, L. and Grever, M.R. (1995) *Life Sci*., **57**, 131-141.

[86] Mi, Q.; Lantvit, D.; Reyes, L.E.; Chai, H.; Zhao, W.; Lee, I.S.; Peraza-Sanchez, S.; Ngassapa, O.; Kardono, L.B.; Riswan, S.; Hollingshead, M.G.; Mayo, J.G.; Farnsworth, N.R.; Cordell, G.A.; Kinghorn, A.D. and Pezzuto, J.M. (2002) *J. Nat. Prod.*, **65**, 842-850.

[87] Mata-Greenwood, E.; Cuendet, M.; Sher, D.; Gustin, D.; Stock, W. and Pezzuto, J.M. (2002) *Leukemia* **16**, 2275-2284.

[88] Cuendet, M.; Christov, K.; Lantvit, D.D.; Deng, Y.; Hedayat, S.; Helson, L.; McChesney, J.D. and Pezzuto, J.M. (2004) *Clin. Cancer Res.*, **10**(3), 1170-1179.

[89] Pisha, E.; Chai, H.; Lee, I.-S.; Chagweder, T.E.; Farnsworth, N.R.; Cordell, G.A.; Beecher, C.W.W.; Fong, H.H.S.; Kinghorn, A.D.; Brown, D.M.; Wani, M.C.; Wall, M.E.; Hieken, T.J.; Das Gupta, T.K. and Pezzuto, J.M. (1995) *Nat. Med*., **1**, 1046-1051.

[90] Shamon, L.; Pezzuto, J.M.; Graves, J.M.; Mehta, R.R.; Wangcharoentrakul, S.; Sangsuwan, R.; Chaichana, S.; Tuchinda, P.; Cleason, P. and Reutrakul, V. (1997) *Cancer Lett.*, **112**, 113-117.

[91] Lee, S.K.; Cui, B.; Mehta, R.R.; Kinghorn, A.D. and Pezzuto, J.M. (1998) *Chem. Biol. Interact*., **115**, 215-228.

[92] Chen, E.X. and Siu, L.L. (2005) *Curr. Pharm. Des*., **11**(2), 265-272.

[93] Li, Q. and Xu, W. (2005) *Curr. Med. Chem. Anti-Canc. Agents*, **5**(1), 53-63.

[94] Segota, E. and Bukowski, R.M. (2004) *Cleve. Clin. J. Med*., **71**(7), 551-560.

[95] Hood L, Heath JR, Phelps ME, Lin B. (2004) *Science*, **306**(5696), 640-643.

[96] McCarty, M.F. (2004) *Integr. Cancer Ther*., **3**, 349-380.

[97] Huang, X.; Chen, S.; Xu, L.; Liu, Y.; Deb, D.K.; Platanias, L.C. and Bergan, R.C. (2005) *Cancer Res*., **65**(8), 3470-3478.

[98] Vantyghem, S.A.; Wilson, S.M.; Postenka, C.O.; Al-Katib, W. and Tuck, A.B. (2005) *Cancer Res*., **65**(8), 3396-3403.

[99] Wang, B.; Li, H.; Yan, H. and Xiao, J.G. (2005) *Methods Find. Exp. Clin. Pharmacol*., **27**(3), 179-184.

[100] Kousidou, O.C.; Mitropoulou, T.N.; Roussidis, A.E.; Kletsas, D.; Theocharis, A.D. and Karamanos, N.K. (2005) *Int. J. Oncol*., **26**(4), 1101-1109.

[101] Ouchi, H.; Ishiguro, H.; Ikeda, N.; Hori, M.; Kubota, Y. and Uemura, H. (2005) *Int. J. Urol*., **12**(1), 73-80.

[102] Su, S.J.; Yeh, T.M.; Chuang, W.J.; Ho, C.L.; Chang, K.L.; Cheng, H.L.; Liu, H.S.; Cheng, H.L.; Hsu, P.Y. and Chow, N.H. (2005) *Biochem. Pharmacol*., **69**(2), 307-318.

[103] Ravindranath, M.H.; Muthugounder, S.; Presser, N. and Viswanathan, S. (2004) *Adv. Exp. Med. Biol*., **546**, 121-165.

[104] Oki, T.; Sowa, Y.; Hirose, T.; Takagaki, N.; Horinaka, M.; Nakanishi, R.; Yasuda, C.; Yoshida, T.; Kanazawa, M.; Satomi, Y.; Nishino, H.; Miki, T. and Sakai, T. (2004) *FEBS Lett*., **577**(1-2), 55-59.

[105] Rimbach, G.; Weinberg, P.D.; de Pascual-Teresa, S.; Alonso, M.G.; Ewins, B.A.; Turner, R. and Minihane, A.M. (2004) *Biochim. Biophys. Acta*, **1670**(3), 229-237.

[106] Bhatia, N. and Agarwal, R. (2001) *Prostate*, **46**, 98-107.

[107] Ye, R.; Bodero, A.; Zhou, B.B.; Khanna, K.K.; Lavin, M.F. and Lees-Miller, S.P. (2001) *J. Biol. Chem*., **276**(7), 4828-4833.

[108] Constantinou, A.I.; Kamath, N. and Murley J.S. (1998) *Eur. J. Cancer*, **34**, 1927-1934.

[109] Weber, G.; Shen, F.; Prajda, N.; Yang, H.; Li, W.; Yeh, A.; Csokay, B.; Olah, E. and Look, K.Y. (1997) *Adv. Enzyme Regul*., **37**, 35-55.

[110] Wang, S.; DeGroff, V.L. and Clinton SK. (2003) *J. Nutr*., **133**(7), 2367-2376.

[111] Valachovicova, T.; Slivova, V.; Bergman, H.; Shuherk, J. and Sliva, D. (2004) *Int. J. Oncol*., **25**(5), 1389-1395.

[112] Sarkar, F.H. and Li, Y. (2003) *Cancer Invest*., **21**(5), 744-757.

[113] Barnes, S.; Sfakianos, J.; Coward, L. and Kirk, M. (1996) *Adv. Exp. Med. Biol*., **401**, 87-100.

[114] Baliga, M.S.; Meleth, S. and Katiyar, S.K. (2005) *Clin. Cancer Res*., **11**(5), 1918-1927.

[115] Pellecchia, M. and Reed, J.C. (2004) *Curr. Pharm. Des*., **10**(12), 1387-1398.

[116] Kazi, A.; Smith, D.M.; Daniel, K.; Zhong, S.; Gupta, P.; Bosley, M.E. and Dou, Q.P. (2002) *In vivo*, **16**(6), 397-403.

[117] Siddiqui, I.A.; Adhami, V.M.; Afaq, F.; Ahmad, N. and Mukhtar, H. (2004) *J. Cell Biochem*., **91**(2), 232-242.

[118] Kuhn, D.J.; Burns, A.C.; Kazi, A. and Dou, Q.P. (2004) *Biochim. Biophys. Acta*, **1682**(1-3), 1-10.

[119] Leone, M.; Zhai, D.; Sareth, S.; Kitada, S.; Reed, J.C. and Pellecchia, M. (2003) *Cancer Res*., **63**(23), 8118-8121.

[120] Saleem, M.; Adhami, V.M.; Siddiqui, I.A. and Mukhtar, H. (2003) *Nutr. Cancer*, **47**(1), 13-23.

[121] Kazi, A.; Wang, Z.; Kumar, N.; Falsetti, S.C.; Chan, T.H. and Dou, Q.P. (2004) *Anticancer Res*., **24**(2B), 943-954.

[122] Hastak, K.; Gupta, S.; Ahmad, N.; Agarwal, M.K.; Agarwal, M.L. and Mukhtar, H. (2003) *Oncogene*, 22, 4851-4859.

[123] Chung, J.H.; Han, J.H.; Hwang, E.J.; Seo, J.Y.; Cho, K.H.; Kim, K.H.; Youn, J.I. and Eun, H.C. (2003) *FASEB J*., **17**, 1913-1915.

[124] Bhattacharyya, A.; Choudhuri, T.; Pal, S.; Chattopadhyay, S.K.; Datta, G.; Sa, G. and Das, T. (2003) *Carcinogenesis* (*Lond*.), **24**, 75-80.

[125] Vergote, D.; Cren-Olive, C.; Chopin, V.; Toillon, R.A.; Rolando, C.; Hondermarck, H. and Le Bourhis, X. (2002) *Breast Cancer Res. Treat.*, **76**, 195-201.

[126] Masuda, M. and Suzui M. (2001) *Clin. Cancer Res*., **7**, 4220-4229.

[127] Chung, L.Y.; Cheung, T.C.; Kong, S.K.; Fung, K.P.; Choy, Y.M.; Chan, Z.Y. and Kwok, T.T. (2001) *Life Sci*., **68**, 1207-1214.

[128] Singh, R.P. and Agarwal, R. (2004) *Mutat. Res*., **555**(1-2), 21-32.

[129] Singh, R.P. and Agarwal, R. (2004) *Curr. Cancer Drug Targets*, **4**(1), 1-11.

[130] Nkondjock, A.; Ghadirian, P.; Johnson, K.C. and Krewski, D. (2005) *J. Nutr*., **135**(3), 592-597.

[131] Tang, L.; Jin, T.; Zeng, X. and Wang, J.S. (2005) *J. Nutr*., **135**(2), 287-290.

[132] Ansari, M.S. and Gupta, N.P. (2004) *Urol. Oncol*., **22**(5), 415-420.

[133] Nahum, A.; Hirsch, K.; Danilenko, M.; Watts, C.K.; Prall, O.W.; Levy, J. and Sharoni, Y. (2001) *Oncogene*, **20**(26), 3428-3436.

[134] Barber, N.J. and Barber, J. (2002) *Prostate Cancer Prostatic Dis.* **5**(1), 6-12.

[135] Karas, M.; Amir, H.; Fishman, D.; Danilenko, M.; Segal, S.; Nahum, A.; Koifmann, A.; Giat, Y.; Levy, J. and Sharoni Y. (2000) *Nutr. Cancer*, **36**(1), 101-111.

[136] Giovannucci, E. (1999) *J. Natl. Cancer Inst*., **91**(4), 317-331.

[137] Dickinson, D.A.; Iles, K.E.; Wigley, A.F. and Forman, H.J. (2004) *Methods Enzymol*., **378**, 302-318.

[138] Lin JK. (2004) *Arch. Pharm. Res.*, **27**(7), 683-692.

[139] Narayan, S. (2004) *J. Mol. Histol*., **35**(3), 301-307.

[140] Aggarwal, B.B.; Kumar, A. and Bharti, A.C. (2003) *Anticancer Res.*, **23**(1A), 363-398.

[141] Chauhan, D.P. (2002) *Curr. Pharm. Des.*, **8**(19), 1695-1706.

[142] Leu, T.H. and Maa, M.C. (2002) *Curr. Med. Chem. Anti-Canc. Agents*, **2**(3), 357-370.

[143] Lin, J.K.; Pan, M.H. and Lin-Shiau, S.Y. (2000) *Biofactors*, **13**(1-4), 153-158.

[144] Yuri. T.; Danbara, N.; Tsujita-Kyutoku, M.; Kiyozuka, Y.; Senzaki, H.; Shikata, N.; Kanzaki, H. and Tsubura, A. (2004) *Breast Cancer Res. Treat*., **84**(3), 251-260.

[145] Xu, M.; Floyd, H.S.; Greth, S.M.; Chang, W.C.; Lohman, K.; Stoyanova, R.; Kucera, G.L.; Kute, T.E.; Willingham, M.C. and Miller, M.S. (2004) *Toxicol. Appl. Pharmacol*., **195**(2), 232-246.

[146] Loutrari, H.; Hatziapostolou, M.; Skouridou, V.; Papadimitriou, E.; Roussos, C.; Kolisis, F.N. and Papapetropoulos, A. (2004) *J. Pharmacol. Exp. Ther*., **311**(2), 568-575.

[147] Clark, S.S.; Zhong, L.; Filiault, D.; Perman, S.; Ren, Z.; Gould, M. and Yang, X. (2003) *Clin. Cancer Res*., **9**(12), 4494-4504.

[148] Crowell, P.L. (1999) *J. Nutr*., **129**(3), 775S-778S.

[149] Belanger, J.T. (1998) *Altern. Med. Rev*., **3**(6), 448-457.

[150] Crowell, P.L. (1997) *Breast Cancer Res. Treat*., **46**(2-3), 191-197.

[151] Wolter, F.; Ulrich, S. and Stein, J. (2004) *J. Nutr*., **134**(12), 3219-3222.

[152] Aggarwal, B.B.; Bhardwaj, A.; Aggarwal, R.S.; Seeram, N.P.; Shishodia, S. and Takada, Y. (2004) *Anticancer Res*., **24**(5A), 2783-2840.

[153] Kundu, J.K. and Surh, Y.J. (2004) *Mutat. Res*., **555**(1-2), 65-80.

[154] Granados-Soto, V. (2003) *Drug News Perspect*., **16**(5), 299-307.

[155] Aziz, M.H.; Kumar, R. and Ahmad, N. (2003) *Int. J. Oncol*., **23**(1), 17-28.

[156] Cal. C.; Garban, H.; Jazirehi, A.; Yeh, C.; Mizutani, Y. and Bonavida, B. (2003) *Curr. Med. Chem. Anti-Canc. Agents*, **3**(2), 77-93.

[157] Bhat, K.P. and Pezzuto, J.M. (2002) *Ann. N.Y. Acad. Sci*., **957**, 210-229.

[158] Cos, P.; De Bruyne, T.; Hermans, N.; Apers, S.; Berghe, D.V. and Vlietinck AJ. (2004) *Curr. Med. Chem.*, **11**(10), 1345-1359.

[159] Bagchi, D.; Bagchi, M.; Stohs, S.; Ray, S.D.; Sen, C.K. and Preuss, H.G. (2002) *Ann. N.Y. Acad. Sci*., **957**, 260-270.
[160] Manson, M.M.; Farmer, P.B.; Gescher, A. and Steward, W.P. (2005) *Recent Results Cancer Res*., **166**, 257-275.
[161] Milner, J.A. (2004) *J. Nutr.* **134**(9), 2492S-2498S.
[162] Aggarwal, B.B.; Takada, Y. and Oommen, O.V. (2004) *Expert Opin. Investig. Drugs*, **13**(10), 1327-1338.
[163] Milner, J.A.; McDonald, S.S.; Anderson, D.E. and Greenwald, P. (2001) *Nutr. Cancer*, **41**(1-2), 1-16.
[164] Greenwald, P.; Milner, J.A.; Anderson, D.E. and McDonald, S.S. (2002) *Cancer Metastasis Rev*., **21**, 217-230.
[165] Kingston, D.G.I. (1996) in *The Practice of Medicinal Chemistry*, (Wermuth, C.G., Ed.), Academic Press, London.
[166] Szostak, J.W. (1997) *Chem. Rev.*, **97**, 347-348.
[167] Leach, A.R. and Hann, M.M. (2000) *Drug Discov. Today*, **5**, 326-336.
[168] Verdine, G.L. (1996) *Nature*, **384**, 11-13.
[169] Henkel, T.; Brunne, R.M.; Müller, H. and Reichel F. (1999) *Angew. Chem. Int. Ed.*, **38**, 643-647.
[170] Feher, M. and Schmidt, J.M. (2003) *J. Chem. Inf. Comput. Sci*., **43**, 218-227.
[171] Bredel, M. and Jacoby, E. (2004) *Nat. Rev. Genet.*, **5**, 262-275.

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