Chapter 2
The National Cancer Institute and Natural Product-Based Drug Discovery in Africa

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Abbreviations

CA1P	Combretastatin A-1 phosphate
CA4P	Combretastatin A-4 phosphate
CBD	United Nations Convention on Biological Diversity
CNARP	Centre National D'Applications des Recherches Pharmaceutiques, Madagascar
CSIR	South African Council for Scientific and Industrial Research
DCTD	Division of Cancer Treatment and Diagnosis
DM1 and DM4	Maytansanoid derivatives conjugated to carrier molecules
DTP	Developmental Therapeutics Program, formerly Cancer Chemotherapy National Service Center (CCNSC)
FDA	US Food and Drug Administration
HIV	Human Immunodeficiency Virus
LOC	NCI Letter of Collection
MaB	Monoclonal Antibody
MDR	Multidrug Resistance
MBG	Missouri Botanical Garden
MOU	NCI Memorandum of Understanding

The opinions expressed in this chapter are those of the authors, not necessarily those of the US government.

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NCI	US National Cancer Institute
NIDDK	National Institute for Diabetes Digestive and Kidney Diseases, NIH
NIH	US National Institutes of Health
NPB	DTP Natural Products Branch
NPR	Natural Products Repository Frederick, Maryland, USA
OTT	NIH Office of Technology Transfer
SCG	Source-Country Government
SCO	Source-Country Scientific Organization
USDA	US Department of Agriculture

2.1 Background

The United States National Cancer Institute (NCI; http://www.nci.nih.gov) was established in 1937, its mission being "to provide for, foster and aid in coordinating research related to cancer." In 1955, NCI set up the Cancer Chemotherapy National Service Center (CCNSC) to coordinate a national, voluntary cooperative cancer chemotherapy program, involving the procurement of drugs, screening, preclinical studies, and clinical evaluation of new agents. The responsibility for drug discovery and preclinical development at NCI now rests with the Developmental Therapeutics Program (DTP; http://dtp.nci.nih.gov); subsequent clinical development, generally up through phase II human trials, is conducted by its companion program, the Clinical Trials Evaluation Program (CTEP; http://ctep.cancer.gov), both being major components of the Division of Cancer Treatment and Diagnosis (DCTD). Thus, for the past 50 years, NCI has provided resources for the preclinical screening and clinical development of compounds and materials submitted by public and private scientists and institutions worldwide and has played a major role in the discovery and development of many of the available commercial and investigational anticancer agents.

During this period, more than 500,000 chemicals, both synthetic and pure natural products, have been screened for antitumor activity using a variety of screening methods. These have ranged from in vivo studies against murine tumors, through human tumor xenografts in immunodeficient mice, to isolated human tumor cell lines and molecular targets expressed in a variety of formats, with the initial systems changing over time. The success of this effort has depended on close collaboration with organizations worldwide, and international collaboration continues to be an important feature of the NCI programs [1].

2.1.1 Achievements: 1955–1982

While most of the materials initially screened were pure compounds of synthetic origin, the program also recognized that natural products were an excellent source

of complex chemical structures with a wide variety of biological activities. The original plant collections from 1960 to 1982 were performed by the US Department of Agriculture (USDA) through an interagency agreement with NCI and involved the random collection of over 35,000 plant samples, mainly from temperate regions. In this period, marine invertebrates were generally collected by academic investigators, mainly funded through grants from the NCI, while microbial samples were obtained from pharmaceutical companies and research institutes, such as the Institute of Microbial Chemistry in Japan, some of which were funded through contracts with the NCI. From 1960 to 1982 [i.e., more than 30 years before the United Nations Convention on Biological Diversity (CBD) to 10 years before], over 180,000 microbial-derived, some 16,000 marine organism-derived, and over 114,000 plant-derived extracts were screened for antitumor activity by the NCI, and as mentioned above, a number of clinically effective chemotherapeutic agents have been developed [2, 3].

2.1.2 Contract Collections (1986–Present): The NCI Letter of Collection

After a lapse in funding from 1982 to 1986, the systematic collection of marine invertebrates and terrestrial plants resumed, coordinated by the DTP Natural Products Branch (NPB; http://dtp.nci.nih.gov/branches/npb/index.html). Marine organism collections originally focused in the Caribbean and Australasia, but, in 1992, were expanded to the Central and Southern Pacific and to the Indian Ocean (off East and Southern Africa) through a contract with the Coral Reef Research Foundation, which is based in Palau in Micronesia. With the renewal of the contract in 2002, collections are now performed worldwide. Terrestrial plant collections have been carried out in over 25 countries in tropical and subtropical regions worldwide through contracts with the Missouri Botanical Garden (MBG) in Africa and Madagascar, the New York Botanical Garden (1986–1996) in Central and South America, the University of Illinois at Chicago in Southeast Asia, and the Morton Arboretum and World Botanical Associates in the United States mainland and territories. Over 60,000 plant samples have been collected, and the permanent repository of over 120,000 extracts is intended to be a resource of potential agents for the treatment all human diseases.

NCI collection contractors are required to obtain all necessary permits, including visas, collecting, shipping, and export permits, from the appropriate source-country agencies or departments. The NCI provides contractors with the NCI Letter of Collection (LOC; http://dtp.nci.nih.gov/branches/npb/agreements.html; http://ttc.nci.nih.gov/forms/) for transmission to the appropriate source-country authorities and scientific organizations (SCOs). The LOC states NCI's willingness to collaborate with local scientists and/or authorities in the discovery and development of novel drugs from organisms (plants, marine invertebrates, microbes) collected in their country and/or

Table 2.1 African countries with which NCI had a letter of collection agreement

Source country	Source-country organization and date of agreement
Gabon	Centre National de la Recherche Scientifique et Technologique (CENAREST), Libreville, 1993
Ghana	University of Ghana, Legon, 1993
Madagascar	Centre National D'Applications des Recherches Pharmaceutiques, Antananarivo, 1990
Tanzania	Traditional Medicine Research Institute, Muhumbili University College of Health Services, University of Dar es Salaam, 1991
Tanzania	Amended agreement signed with The Institute of Traditional Medicine, Muhumbili University College of Health Services, 2009

territorial waters, and, if requested, the NCI will enter into formal agreements based on the LOC with the relevant source-country government agency or organization. LOC agreements have been signed with four African countries (Table 2.1), and it is notable that the first agreement with any country worldwide was signed with CNARP, Antananarivo, in 1990, 2 years prior to the signing of the CBD.

Collections by MBG were performed in Cameroon and the Central African Republic without the finalization of a formal LOC agreement, but the authorities in these two countries were fully aware of the terms of the LOC and granted the necessary permits for MBG collections without requiring a formal agreement. In this respect, the NCI is totally committed to the terms of the LOC irrespective of whether or not a formal agreement has been signed [4]. This absence of formal agreements was not due to any lack of effort on the part of the MBG and/or NCI staff to solicit formal agreements; indeed, in the case of Cameroon, NPB staff interacted extensively with government representatives and scientists, both in Cameroon and during NCI-sponsored visits to NCI and MBG (Table 2.2). The purpose of these visits was to provide opportunities for source-country officials and scientists to observe the NCI drug-discovery facilities and the processes to which their raw materials are subjected, and to discuss collaboration in the drug-discovery process. From 1989 to 2001, 18 officials and scientists from African countries visited NCI one or more times for periods of 1–2 weeks, either to discuss participation in NCI contract collections or direct collaboration in the drug-discovery process (Table 2.2).

2.1.3 Source-Country Collaboration

In carrying out these collections, NCI contractors work closely with qualified organizations in each of the source countries. Botanists and marine biologists from source-country organizations (SCOs) collaborate in field collection activities and taxonomic identification, and their knowledge of local species and conditions is indispensable to the success of the NCI collection operations. SCOs, when necessary and relevant, provide facilities for the preparation, packaging, and shipment of

Table 2.2 Short-term (1–2 weeks) African visitors to the USA sponsored by NCI

Year	Visitor	Institution	Country
1989	Dr. Elimweka Mshiu	U. Dar Es Salaam	Tanzania
	Dr. Johnson Jato	U. Yaounde 1	Cameroon
	Dr. Robodo Andriantsiferana	CNARP[a]	Madagascar
	Dr. Feetham Banyikwa	U. Dar Es Salaam	Tanzania
1990	Dr. Rogasian Mahunnah	U. Dar Es Salaam	Tanzania
1991	Dr. Blandine Akendengue	CENAREST[b]	Gabon
	Dr. Johnson Jato	U. Yaounde 1	Cameroon
1992	Dr. Johnson Jato	U. Yaounde 1	Cameroon
	Dr. Ivan Addae-Mensah	U. Ghana, Legon	Ghana
1993	Dr. Lucien Obame	CENAREST[b]	Gabon
	Dr. Lucienne Nze-Ekekang	CENAREST[b]	Gabon
1994	Dr. J. Rajaonarivony	CNARP[a]	Madagascar
	Dr. Johnson Jato	U. Yaounde 1	Cameroon
	Dr. Thomas Tata	Ministry of the Environment	Cameroon
1995	Dr. J. Rajaonarivony	CNARP[a]	Madagascar
	Mr. J. Edou	Office of the Prime Minister	Cameroon
	Dr. Johnson Jato	U. Yaounde 1	Cameroon
	Dr. T. Mbenkum	Ministry of the Environment	Cameroon
	Dr. G. Chavanduka	U. Zimbabwe	Zimbabwe
	Dr. P. Mashava	U. Zimbabwe	Zimbabwe
	Mr. R. Chadwick	Legal rep., U. Zimbabwe	Zimbabwe
	Dr. W. Phillips	U. Ghana, Legon	Ghana
1997	Dr. P. Mashava	U. Zimbabwe	Zimbabwe
2001	Dr. M. Andriantsoa	CNARP[a]	Madagascar

[a]CNARP: Centre National D'Appliques Recherches Pharmaceutique, Madagascar
[b]CENAREST: Centre National de la Recherches Scientifique et Technologique, Gabon

the samples to the NCI's Natural Products Repository (NPR) in Frederick, Maryland. In a significant number of cases, these interactions have materially aided the procurement of both the initial collection permits and, most importantly, the specific export documentation required by the country of origin.

The collaboration between the SCOs and the NCI collection contractors, in turn, provides support for expanded research activities by source-country biologists. The deposition of a voucher specimen of each species collected in the national herbarium or repository expands source-country documentation of their biota. NCI contractors also provide training opportunities for local personnel through conducting workshops and presentation of lectures, both in-country and at the contractor's US facilities. In the context of plant collections in African countries, during the contract cycle from 1996 to 2001, MBG offered one-month curatorial workshops at their facilities in St. Louis, Missouri in May 1999 and March 2001. Through its contract with MBG, the NCI supported the attendance of seven botanists from Madagascar, Ghana, Tanzania, and Zambia, and participants were instructed in collections management, botanical research methodology, biodiscovery, conservation, and global information systems.

Table 2.3 Long-term (1–12 months) African visiting scientists sponsored under the auspices of the NCI LOC

Year	Visitor	Home institution	Country	US host institution
1990	Dr. Z. Mbwambo	U. Dar Es Salaam	Tanzania	NCI
1993	Mr. C. Mutayabarwa	U. Dar Es Salaam	Tanzania	NCI
1994	Dr. R. Andriamaharavo	U. Antananarivo	Madagascar	NIDDK[a], NIH
	Dr. J. Jato	U. Yaounde 1	Cameroon	NCI
1995	Dr. W. Phillips	U. Ghana, Legon	Ghana	VPISU[b]
1996	Dr. V. Rasimison	CNARP[c]	Madagascar	Washington U., St. Louis
1997	Dr. Sadri Said	U. Dar Es Salaam	Tanzania	U. Oklahoma
	Ms. J. Ropivia	CENAREST[d]	Gabon	UIC[e]
2000	Dr. M. Lamidi	CENAREST[d]	Gabon	U. Mississippi
2002	Dr. R. Andriamaharavo	U. Antananarivo	Madagascar	NIDDK[a], NIH
2003	Dr. Ladislaus Mdee	U. Dar es Salaam	Tanzania	UIC[e]
2004	Dr. Johnson Jato (Fulbright Scholar)	Bamenda U. Sci. and Technology	Cameroon	NCI

[a]*NIDDK* National Institute for Diabetes, Digestive and Kidney Diseases, NIH
[b]*VPISU* Virginia Polytechnic Institute and State University
[c]*CNARP* Centre National D'Appliques Recherches Pharmaceutique, Madagascar
[d]*CENAREST* Centre National de la Recherches Scientifique et Technologique, Gabon
[e]*UIC* University of Illinois at Chicago

In addition, through its LOC and agreements based upon it, the NCI has invited 10 African scientists nominated by SCOs to visit its facilities, or equivalent facilities in other approved US organizations, for 1–12 months to participate in collaborative natural products research involving the screening and bioassay-directed fractionation of extracts (Table 2.3). The LOC also dictates benefit-sharing and use of source-country resources in the event of the licensing and development of a promising drug candidate. Successful licensees are required to negotiate agreements with source-country government (SCG) agencies or SCOs, dictating terms of collaboration and compensation. The terms apply irrespective of whether the potential drug is the actual isolated natural product or a compound structurally based upon the isolate, a synthetic material for which the natural product provided a key development lead, or a method of synthesis or use of any aforementioned isolate, product, or material. The percentage of royalties negotiated as payment varies depending upon how closely the marketed drug relates to the originally isolated product. The first milestone in the licensing agreement is that a signed agreement must be presented to the NIH's Office of Technology Transfer (OTT), the group within NIH that formally licenses all NIH patents, within 1 year of the initial granting of the license.

The original formulation of the NCI policies for collaboration and benefit-sharing embodied in the LOC predated the drafting of the CBD (http://www.biodiv.org/convention/articles.asp) by at least 4 years, and as noted in Sect. 2.1.2, the first agreement was signed with CNARP, Madagascar, in 1990.

Table 2.4 MOU between NCI and African organizations: direct collaborations

Country	Organization and date of MOU
Zimbabwe	Zimbabwe National Traditional Healers Association (ZINATHA), 1994
S. Africa	Council for Scientific and Industrial Research (CSIR), Division of Food, Biological and Chemical Technologies (BIO/CHEMTEK), 1996
S. Africa	Rhodes University, 1998

Table 2.5 Long-term (1–12 months) African visiting scientists sponsored by NCI under the auspices of the MOU

Year	Visitor	Home institution	Country	US host institution
1994	Dr. P. Mashava	U. Zimbabwe	Zimbabwe	NCI
1997	Ms. H. Van Vuuren	CSIR, Pretoria	S. Africa	NCI
1999	Dr. P. Mashava	U. Zimbabwe	Zimbabwe	NCI
1999	Dr. M. Davies-Coleman[a]	Rhodes University	S. Africa	NCI

[a]Visiting scientist supported through NIH Research Fellowship to NCI Laboratory of Drug Discovery, Research and Development

2.1.4 Direct Collaboration with Source-Country Organizations: The NCI Memorandum of Understanding

As mentioned in Sect. 2.1.2, the collections of plants and marine organisms have been carried out in over 25 countries worldwide through contracts with qualified US botanical and marine biological organizations working in close collaboration with qualified SCOs, and all collections are performed subject to the terms of the NCI Letter of Collection. Particularly in the area of plant-related studies, source-country scientists and governments are becoming increasingly committed to performing more of the drug-discovery operations in-country, as opposed to simply exporting raw materials. The NCI has recognized this fact for several years, and contract collections of plants are now being de-emphasized in favor of establishing direct collaborations with qualified organizations in the source countries where the necessary expertise and infrastructure exist.

The NCI has established collaborative agreements [Memoranda of Understanding (MOU); http://dtp.nci.nih.gov/branches/npb/agreements.html; http://ttc.nci.nih.gov/forms/] with over 20 SCOs suitably qualified to perform in-country processing, including three from Southern Africa (Table 2.4).

In establishing these agreements, the NCI undertakes to abide by the same policies of collaboration and compensation as specified in the LOC. Depending on the availability of the necessary resources, NCI also assists the SCOs in establishing their own drug-discovery programs through training in techniques of antitumor screening and natural product isolation. NCI has sponsored long-term visitors from 18 countries worldwide since 1988 for such purposes of collaboration and training, including three from Southern Africa (Table 2.5).

It is anticipated that the discovery of novel anticancer drugs will be performed by the SCO at its own expense, with assistance from the NCI in terms of free secondary in vitro and in vivo testing. All results from such secondary testing are considered the sole intellectual property of the SCO since the NCI regards such testing as a routine service to the scientific community and can be used by the SCO to apply for patents covering promising inventions. The NCI will commit its resources to collaborating with the SCO in the preclinical and clinical development of any SCO-discovered drug which meets the NCI selection criteria and will make a sincere effort to transfer any knowledge, expertise, and technology developed during such collaboration to the SCO, subject to the provision of mutually acceptable guarantees for the protection of intellectual property associated with any patented technology.

2.1.5 NCI Screening Agreement

As noted in Sect. 2.1, the NCI has played a major role in the discovery and development of many of the available commercial and investigational anticancer agents. Organizations or individuals wishing to have pure compounds tested in the NCI drug screening program, such as pharmaceutical and chemical companies or academic research groups worldwide, may submit their compounds for free testing through an online submission process which can be accessed at http://dtp.nci.nih.gov/docs/misc/common_files/submit_compounds.html. A screening agreement (http://dtp.nci.nih.gov/docs/misc/common_files/canagr.html) may be signed with the NCI Division of Cancer Treatment and Diagnosis (DCTD) which includes terms stipulating confidentiality and levels of collaboration in the drug development process. Should a compound show promising anticancer activity in the routine screening operations, the NCI may propose the establishment of a more formal collaboration for further development, such as a Cooperative Research and Development Agreement (CRADA) or a Clinical Trial Agreement (CTA) (http://ttc.nci.nih.gov/forms/).

2.2 African Plant-Derived Anticancer Agents: Recent Developments

Plants have a long history of use in the treatment of cancer. Hartwell [5], in his review of plants used against cancer, lists more than 3,000 plant species that have reportedly been used in the treatment of cancer. In many instances, however, the "cancer" is undefined, or reference is made to conditions such as "hard swellings," abscesses, calluses, corns, warts, polyps, or tumors. Many of these claims for efficacy in the treatment of cancer, however, should be viewed with skepticism

because cancer, as a specific disease entity, is likely to be poorly defined in folklore and traditional medicine [6]. This is in contrast to other plant-based therapies used in traditional medicine for the treatment of afflictions such as malaria and cutaneous fungal infection, which are more easily defined and which are prevalent in the regions where traditional medicine systems are used extensively. Of the plant-derived anticancer drugs in clinical use, among the best known are the so-called vinca alkaloids, vinblastine (VLB), and vincristine (VCR), isolated from the Madagascar periwinkle *Catharanthus roseus*. *C. roseus* has been used by various cultures for the treatment of diabetes, and vinblastine and vincristine were first discovered during an investigation of the plant as a source of potential oral hypoglycemic agents. Their discovery, therefore, may be indirectly attributed to the observation of an unrelated medicinal use of the source plant. It is interesting to note that, though the plant was originally endemic to Madagascar, the samples used in the discovery of VLB and VCR were collected in Jamaica and the Philippines. More recent semisynthetic analogues of these agents are vinorelbine (VRLB) and vindesine (VDS). These agents are primarily used in combination with other cancer chemotherapeutic drugs for the treatment of a variety of cancers.

Other important plant-derived, clinically used agents are etoposide and teniposide, which are semisynthetic derivatives of the natural product epipodophyllotoxin; the taxanes, paclitaxel (Taxol®) and docetaxel (Taxotere); topotecan (hycamptamine) and irinotecan (CPT-11) semisynthetically derived from camptothecin; and homoharringtonine. These and other important anticancer agents in clinical and preclinical development have been comprehensively reviewed, and interested readers should consult this review for details [2].

2.2.1 The Combretastatins: Models for Combinatorial Chemistry

The combretastatins were isolated from the South African "bush willow," *Combretum caffrum* (Eckl. & Zeyh.) Kuntze, collected in Southern Africa in the 1970s for the NCI by the United States Department of Agriculture (USDA), working in collaboration with the Botanical Research Institute of South Africa. These collections were part of a random collection program aimed at the discovery of novel anticancer agents. Species of the *Combretum* and *Terminalia* genera, both of which belong to the Combretaceae family, are used in African and Indian traditional medicine for the treatment of a variety of diseases, including hepatitis and malaria. Several *Terminalia* species have reportedly been used in the treatment of "cancer."

The combretastatins are a family of stilbenes which act as anti-angiogenic agents, causing vascular shutdown in tumors which leads to tumor necrosis [7, 8]. Two water-soluble analogues, combretastatin A-4 phosphate (CA4P; fosbretabulin disodium) and combretastatin A-1 phosphate (CA1P; OXi4503) (Fig. 2.1) have advanced into clinical trials under the sponsorship of Oxigene, Inc. To date, there have been 12 clinical trials involving CA4P against a variety of cancers, mostly in combination with other agents such as carboplatin and paclitaxel, as well as

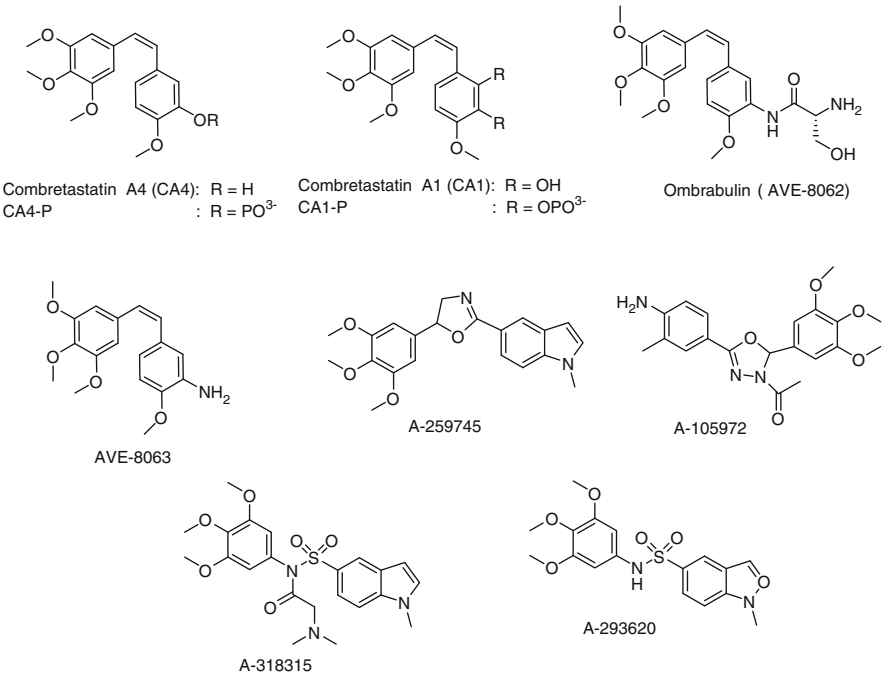

Fig. 2.1 Some combretastatin analogues and mimics

radiation treatment. Currently, two phase II trials are ongoing, one against non-small cell lung cancer (NSCLC) in combination with carboplatin, paclitaxel, and bevacizumab and the other against anaplastic thyroid cancer (ATC) in combination with carboplatin and paclitaxel. The clinical experience with CA4P has been reviewed [9]. Combretastatin A1P, like CA4P, shows excellent activity in preclinical studies, and a phase I clinical trial is under way, evaluating the safety and tolerability in patients with solid tumors, while a phase Ib/II trial is assessing the safety, tolerability, and efficacy, specifically against solid tumors growing in the liver [8]; in addition, patients are currently (June, 2012) being recruited for a phase I trial against Acute Myelogenous Leukemia (AML) and Acute Myelodysplastic Syndromes (AMS) [http://www.clinicaltrials.gov/ct2/results?term=cancer+combretastatins].

CA4P has also shown promising activity against ophthalmological diseases such as myopic macular degeneration (MMD), where all 23 patients in a phase II clinical trial achieved the primary endpoint of the trial, stabilization of vision. Parallel phase I/II clinical trials using CA4P for the treatment of exudative age-related macular degeneration (AMD) are also in progress [8, 10].

A number of combretastatin analogues and mimics are also being developed [8, 11]. The combretastatin chemical class has served as a model for the synthesis of a host of analogues containing the essential trimethoxy aryl moiety linked to substituted aromatic moieties through a variety of two or three atom bridges, including heterocyclic rings and sulfonamides. Some of these are shown in

Fig. 2.1. This is an impressive display of the power of a relatively simple natural product structure to spawn a prolific output of medicinal and combinatorial chemistry. One of these analogues, ombrabulin (AVE8062), is currently in a phase III clinical trial against advanced soft tissue sarcoma [http://www.clinicaltrials.gov/ct2/results?term=cancer+combretastatins].

2.2.2 *Maytansine: Targeting Toxic Natural Products*

A recurring liability of natural products, at least in the area of cancer chemotherapy, is that, although many are generally very potent cytotoxins cell growth inhibitors, they have limited solubility in aqueous solvents and exhibit considerable toxicity, often resulting in a narrow therapeutic index. These factors have led to the demise of a number of pure natural products as promising leads, including the plant-derived agents bruceantin and maytansine. An alternative strategy for the utilization of such agents is to investigate their potential as "warheads" attached to monoclonal antibodies specifically targeting epitopes on the tumor of interest. The promise of this approach to cancer therapy has been the subject of several reviews, and readers are referred to them for further details of developments in this area [12–16].

A promising candidate for such an approach is maytansine [17–19]. Maytansine (Fig. 2.2) was isolated in extremely low yield (2×10^{-5}% based on plant dry weight) in the early 1960s from the Ethiopian plant *Maytenus serrata* (Hochst. Ex A. Rich.) Wilczek (Celastraceae family) collected for the NCI as part of a random collection program performed through a collaboration with the US Department of Agriculture (USDA). The novel structure and very potent in vitro activity of maytansine prompted great interest in pursuing further preclinical, and possible clinical, investigation. In order to obtain sufficient quantities, other species of *Maytenus* and related members of the Celastraceae family were surveyed; *Maytenus buchanii* (Loes) R. Wilczek collected in Kenya was shown to produce a seven times higher yield, while *Putterlickia verrucosa* (E. May ex Sonder) Szyszyl collected in South Africa proved to be the richest source, with a yield eight times that from *Maytenus buchanii*. Given the greater ease of large-scale collections of *M. buchanii*, however, a large recollection (about 9,000 kg) was undertaken in Kenya in 1976. Even given the improvement in yields, these remained extremely low, but the extreme potency of maytansine in testing against cancer cell lines permitted the production of sufficient quantities for pursuit of preclinical and clinical development. In appreciation of the collaboration of Kenyan authorities in the recollection process, the NCI donated supplies of the antileukemic drugs, vinblastine and vincristine, and preliminary studies aimed at the cultivation of *M. buchanii* were initiated [private communication, USDA Program Coordinator Dr. Robert Perdue (deceased, July 2011)].

Unfortunately, the very promising activity observed in preclinical animal testing of maytansine failed to translate to significant efficacy in human clinical trials, and it was dropped from further study in the early 1980s. Related compounds, the ansamitocins, were subsequently isolated from a microbial source, the

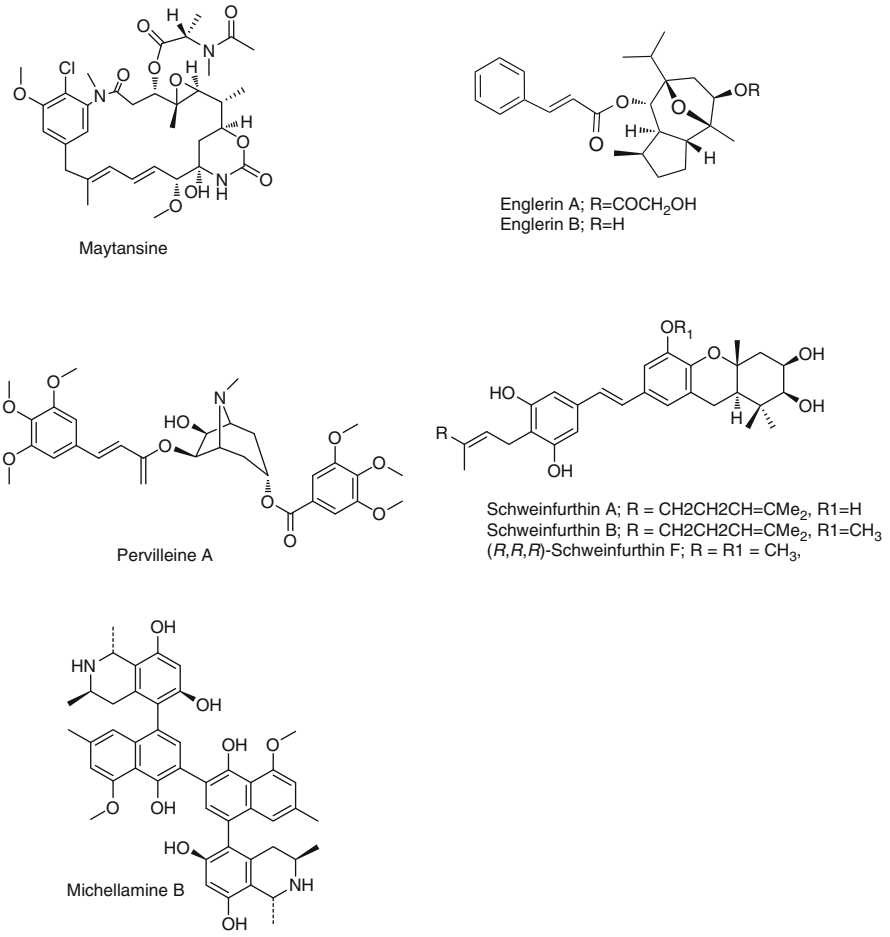

Fig. 2.2 Maytansine, englerins, schweinfurthins, pervilleine A, michellamine B

actinomycete *Actinosynnema pretiosum*, posing the question as to whether the maytansines are actually plant products or are produced through an association between a microbial symbiont and the plant. While this remains a topic of continued interest, the microbial production of the closely related ansamitocins allowed for easier production of larger quantities of this class of compounds, and this factor, together with their extreme potency, stimulated heightened interest in pursuing their development.

Maytansanoid derivatives, DM1 and DM4, can be conjugated through either thioether or disulfide linkages with various monoclonal antibodies (mAbs) targeting a variety of cancers [17, 19]. Conjugates have been prepared with huC242, a mAb directed against the *muc*1 epitope expressed in pancreatric, biliary, colorectal, and gastric cancers. HuC242-DM4 has undergone a phase I clinical trial for the treatment of CanAg-expressing tumors such as carcinomas of the colon and pancreas

[20], and a further phase I trial of this agent is ongoing (April 2011) in patients with inoperable or metastatic colorectal cancer, pancreatic cancer, or other solid tumors (http://www.cancer.gov/drugdictionary/?CdrID=492706). DM4-conjugated anti-Cripto monoclonal antibody, BIIB-015, is in a phase I study in patients with relapsed/refractory solid tumors (http://www.cancer.gov/drugdictionary/?CdrID=596550). Cripto, a member of the EGF-CFC family of growth factor-like molecules, is overexpressed in carcinomas such as those of the breast, ovary, stomach, lung, and pancreas, while not expressed in normal tissues.

Two drugs, trastuzumab (Herceptin) and lapatinib (Tykerb), are currently approved for the treatment of HER2-positive breast cancer, but they are not effective for all patients suffering from this disease. In efforts to address this problem, trastuzumab linked to DM1 through a non-reducible thioether linkage (trastuzumab-MCC-DM1) was shown to be active in trastuzumab-sensitive and trastuzumab-refractory models of HER2-overexpressing cancer and has shown some clinical responses in a phase I clinical trial [21]. Six studies of trastuzumab-MCC-DM1 in patients with HER2-positive forms of breast cancer have been completed (http://www.cancer.gov/clinicaltrials/search/results?protocolsearchid=8826956), and as of April 2011, four trials (http://www.cancer.gov/drugdictionary/?CdrID=564399) are ongoing. These include a phase III trial in patients with HER2-positive, locally advanced or metastatic breast cancer, a phase III trial in combination with pertuzumab in patients with HER2-positive locally advanced or metastatic breast cancer, a phase I study in combination with docetaxel in patients with locally advanced or metastatic HER2-positive breast cancer, and a phase I study in combination with paclitaxel and pertuzumab in patients with HER2-positive locally advanced or metastatic breast cancer.

The current most complete listing of the preclinical and clinical status of these agents is reported in the 2010 review by Lambert [19]. In summary, through the end of 2009, there have been 14 DM1 or DM4 linked agents that have been reported in preclinical through clinical trials. Of these, four currently fall into the preclinical area, while five are listed in phase I trials. These are SAR-3419 from Sanofi-Aventis, IMGN-388 from Immunogen, IMGN-633 (AVE-9633) from Immunogen/Sanofi-Aventis, BT-062 from Biotest-AG, and BIIB-015 from Biotest-Idec. Two more from Immunogen are in phase I/II trials, namely, IMGN-901 (lorvotuzumab mertansine) and IMGN-242. A more recent review in the "News and Analysis" section of the journal Nature Reviews: Drug Discovery indicates that SAR-3419 and IMGN-901 are now in phase II trials [22].

The fourteenth and most advanced of the DM-linked conjugates, which is currently in phase III trials (April 2011), is from Genentech/Roche and is known as T-DM1 or trastuzumab emtansine. As a result of the data from the phase II trial on patients who had failed at least two HER2 + treatments (trastuzumab and the tyrosine kinase inhibitor lapatinib), a biological license application (BLA, equivalent to the NDA application for small molecules) was submitted to the FDA by Genentech for marketing approval as a treatment for HER2+ breast cancer. Details of the agent and results to the end of 2009 were reported by Lambert [19] and in more detail by Niculescu-Duvaz [23]. Further detailed discussions are given in Ref. 17, and a less technical commentary is given in the article by Hughes [22].

2.2.3 *Englerins*

The englerins (Fig. 2.2) are new guaiane sesquiterpenes isolated from the Tanzanian plant *Phyllanthus engleri* Pax collected by Z. Mbwambo and MBG staff in 1989. The extract was prepared at NCI in 1992, and testing of the extract in the NCI 60-cancer cell line screen in 1997 showed selective inhibition of growth of the renal cancer cell lines; however, the extract was not pursued at that point. Bioassay-guided fractionation in 2007 yielded englerins A and B, and englerin A showed 1,000-fold selectivity against six of eight renal cancer cell lines with GI(50) values ranging from 1 to 87 nM [24]. These compounds are being developed in collaboration with scientists from the Institute of Traditional Medicine of Muhimbili University of Health and Allied Services in Dar es Salaam, Tanzania (Table 2.1). While the original LOC with ITM had expired at the time of the discovery, an updated LOC was negotiated in 2009 which permitted a multikilogram recollection of plant material to be made. This single collection yielded gram quantities of englerin A for preclinical development at NCI, which is ongoing.

The outstanding activity of englerin A in the NCI 60 cell screen has led many synthetic chemistry groups to attempt the total synthesis of englerin A. The first total synthesis was reported by the Christmann group from Dortmund, Germany [25]. This work also established the absolute configuration of the natural product. Other groups have since reported different synthetic strategies, including Nicolaou [26], Echavarren [27], and Ma [28]. Very recently, a more efficient process has been developed by Chain, in which englerin A was prepared in eight steps and 20% overall yield from readily available starting materials [29].

Structure–activity relationships have been explored for the englerins by both the Chen [30] and Christmann groups [31]. Notably, replacement of the cinnamate ester with a naphthoate moiety led to improved selectivity [31], while a reverse glycol ester and a lactate ester were worthy of note [30]. 9-Deoxy-englerin A showed significantly reduced activity [32].

The mechanism of englerin A's effect on renal cancer has been proposed to involve protein kinase C agonism, with inhibitory effects on the insulin pathway [33]. Given the dependence of renal cancers on glycolysis, this effect of englerin A may lead to its observed experimental therapeutic effects. The availability of labeled compounds (e.g., fluorescent or biotinylated derivatives) derived from synthetic approaches to englerins will likely shed more light on the mechanistic questions.

2.2.4 *Schweinfurthins*

The schweinfurthins (e.g., schweinfurthins A, B, and F; Fig. 2.2) were isolated in 1996 from the African plant *Macaranga schweinfurthii* Pax, collected in Cameroon in 1987 by MBG. They displayed significant selective activity against central nervous system, renal, and breast cancer cell lines in the NCI 60 cell line anticancer

assay [34]. The spectrum of anticancer activity does not match that of any currently used agent, indicating that these compounds may be acting at a previously unrecognized target or through a novel mechanism. Thus far, ten schweinfurthins (A to J) have been isolated from nature by the NCI and Kingston/Virginia Polytechnic groups [35–37]. While successful scale-up recollections and isolation were undertaken in collaboration with colleagues in Cameroon in 1998–1999 and thereafter [38], the isolation of sufficient quantities of the schweinfurthins from the natural source remains challenging, and synthetic strategies have been developed to provide a reliable source of natural schweinfurthins and synthetic analogues for further biological testing [39, 40]. In the case of schweinfurthin F, total synthesis of the (*R*,*R*,*R*) and (*S*,*S*,*S*) enantiomers and comparisons of spectral data, optical rotations, and bioassay data with those reported for the natural product have resulted in assignment of the natural material as the (*R*,*R*,*R*) isomer [41]. These synthetic efforts are continuing, and most of the naturally occurring schweinfurthins have now been obtained by total synthesis [42–47].

Investigations into the mechanism of action of schweinfurthins have yet to identify a proximate molecular target; however, in glioblastoma cell lines, it appears that a defective neurofibromatosis type 1 (NF1) pathway confers sensitivity [48]. Other recent work has implicated an effect on oxysterol binding proteins and other isoprenoid pathways [48a, 48b].

2.2.5 The Pervilleines: Potential Multidrug Resistance Inhibitors

The resistance to treatment with standard anticancer agents developed by many cancer patients is a serious problem encountered in cancer chemotherapy [49]. Resistance to a drug may develop in a cell population through repeated exposure to treatment with that particular drug, and this cell population may subsequently show broad cross-resistance to other anticancer agents even though it has never been exposed to those agents. This phenomenon is called multidrug resistance (MDR) and has been linked to the presence of the *MDR1* gene which encodes P-glycoprotein (Pgp) which effectively pumps the drugs out of the cell, thereby precluding their antitumor actions. A large number of compounds which reverse this effect in vitro in cell line studies (so-called MDR inhibitors) have been discovered, but their effectiveness in the clinic has been mostly disappointing. Thus, there is a continuing search for more effective MDR inhibitors.

The pervilleines, isolated from *Erythroxylum pervillei* Baillon, collected in Madagascar in 2003 have shown promising MDR activity both in vitro and in vivo [50–52]. Pervilleine A (Fig. 2.2) was selected for preclinical development through a collaboration between an NCI-supported National Cooperative Drug Discovery Group (NCDDG) and the Institut Malgache de Recherches Appliquées. The fact that racemic pervilleine A hydrochloride exhibited only weak cholinergic and adrenergic receptor-mediated activities was considered advantageous for the further development of pervilleine A as a new adjuvant in cancer chemotherapy [53].

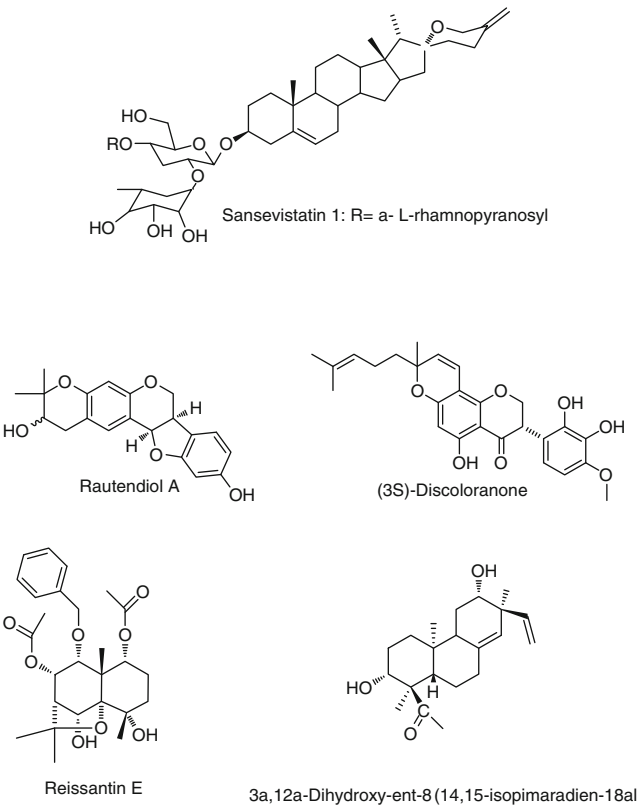

Fig. 2.3 Other novel agents

2.2.6 Other Novel Agents Discovered Through Direct NCI Support

A recent review [54] recorded several other novel antitumor agents discovered through projects directly supported by NCI, either by the early program (1955–1982; Sect. 2.1.1) or by the more recent NCI LOC program (Sects. 2.1.2 and 2.1.3). Sansevistatins 1 (Fig. 2.3) and 2, new cytotoxic spirostanol saponins, were isolated from *Sansevieria ehrenbergii*, initially collected in Kenya in 1966 by the USDA through an interagency agreement with NCI (Sect. 2.1.1) [55]. Several Tanzanian plants collected by the NCI contractor, Missouri Botanical Garden (Sect. 2.1.2), have yielded novel cytotoxic agents. In addition to the englerins (Fig. 2.2) discussed in Sect. 2.2.3, these include new agarofuran sesquiterpenes, reissantins A–E (E; Fig. 2.3), isolated from *Reissantia buchananii* collected in 1989 [56], and rautandiols A (Fig. 2.3) and B, pterocarpans, isolated from *Neorautanenia mitis* collected in 1993 [57]; while the rautandiols did not exhibit significant cytotoxicity, several known rotenone derivatives were isolated as the cytotoxic

constituents. During visits of Tanzanian scientists sponsored by the NCI (Table 2.3) to the University of Illinois at Chicago, two new diterpenes, (3α,12α)-dihydroxy-ent-8(14),15-isopimaradien-18-al (Fig. 2.3), and *trans*-9-acetyl-4,9'-di-*O*-methyl-3'-de-*O*-methyldehydrodiconiferyl alcohol were isolated from *Euphorbia quinquecostata* collected by Z. Mbwambo in 1999 [58], while *Berchemia discolor* collected in 1999 yielded new prenylated flavonoids ((3 *S*)-discoloranone; Fig. 2.3) [59].

2.3 Michellamine B: A Potential Anti-HIV Agent from the Cameroonian Liana, *Ancistrocladus korupensis*

From 1987 to 1996, the NCI tested over 60,000 extracts of natural origin in an in vitro cell-based anti-HIV screen which determined the degree of HIV-1 replication in treated infected lymphoblastic cells versus that in untreated infected control cells. Several plant-derived natural products have shown in vitro activity, and of these, michellamine B is the only anti-HIV agent isolated from an African source to advance into preclinical development.

In 1987, a sample of the leaves of a liana identified as an *Ancistrocladus* species was collected in the Korup region of southwestern Cameroon as part of the NCI contract with Missouri Botanical Garden for collections in Africa and Madagascar. Extracts of the leaves exhibited significant in vitro anti-HIV activity, and the dimeric naphthylisoquinoline alkaloid, michellamine B (Fig. 2.2), was isolated as the active agent [60, 61]. Michellamine B, named after the wife of the chemist who performed the initial isolation, showed in vitro activity against both HIV-1 and HIV-2 and was shown to inhibit human immunodeficiency virus-induced cell killing by at least two distinct mechanisms, acting at an early stage of the HIV life cycle by inhibiting reverse transcriptase as well as at later stages by inhibiting cellular fusion and syncytium formation [62]. Thus, it was selected for preclinical development.

The source plant was later identified as a new species and named *A. korupensis* D. Thomas & Gereau [63]. This new species was found only in and around the Korup National Park, and vine densities were very low, on the order of one large vine per hectare. While fallen leaves contained michellamine B, and their collection provided sufficient biomass for the isolation of enough drug to complete preclinical development [64], it was clear that extensive collections of fresh leaves could pose a possible threat to the limited and sparse wild population.

Thus far, no other *Ancistrocladus* species has been found to contain michellamine B, and investigation of the feasibility of cultivation of the plant as a reliable biomass source was initiated in 1993 through a contract with the Center for New Crops and Plant Products of Purdue University working in close collaboration with the University of Yaounde 1, the World Wide Fund for Nature Korup Project, MBG, Oregon State University, and the NCI-Frederick contractor, Science Applications International Corporation (SAIC). An extensive botanical survey was undertaken, and the

range and distribution of the species were mapped, and dried leaves were analyzed for michellamine B content. Promising plants were resampled for confirmatory analysis, and those showing reproducible high concentrations were targeted for vegetative propagation. A medicinal plant nursery was established for the *A. korupensis* collection near Korup Park Headquarters in Mundemba, and through selection of promising plants from the wild and their subsequent propagation and growth in the nursery, it was demonstrated that michellamine content well above the wild collected average could be produced routinely. In keeping with the NCI policies of collaboration with source countries, all the cultivation studies were performed in Cameroon and involved the local population in the Korup region where the plant was originally discovered.

Based on the observed activity and the efficient formulation of the diacetate salt, the NCI committed michellamine B to advanced preclinical development, but continuous infusion studies in dogs indicated that in vivo effective anti-HIV concentrations could only be achieved at doses close to neurotoxic levels. Thus, despite in vitro activity against an impressive range of HIV-1 and HIV-2 strains, the difference between the toxic dose level and the anticipated level required for effective antiviral activity (the therapeutic index) was small, and NCI discontinued further studies aimed at clinical development. However, the discovery of novel antimalarial agents, the korupensamines, from the same species [65, 66] added further promise for this species. Michellamines have also been reported to show antioxidant activity [67] and inhibition of protein kinase C [68], while michellamine B has been shown to be a potent but nonselective inhibitor of platelet-type 12-human lipoxygenase [69]. Human lipoxygenases have been implicated in a variety of diseases involving inflammation, immune disorders, and various types of cancers [70, 71]. The publication of several synthetic routes to the michellamines [72, 73] should permit the study of structure–activity relationships, thereby increasing the potential for structural optimization to improve anti-HIV and other bioactivities relative to toxicity.

As mentioned in Sect. 2.1.2, despite negotiations with the appropriate authorities, no formal LOC agreement for plant collections was finalized with the Cameroon government; however, collections by MBG were performed with all of the necessary permits issued by the appropriate government departments. Upon the discovery of the anti-HIV activity of extracts of *A. korupensis* and the isolation of michellamine B as the active agent, the Cameroon government established an Intraministerial Committee for Research on *A. korupensis*, under the chairmanship of Mr. J. Edou of the Prime Minister's Office, to monitor progress in both the development of michellamine B and the cultivation project. The cultivation project was carried out with the full permission and cooperation of the Cameroon government, and close contact was maintained with the Intraministerial Committee regarding progress in all aspects of michellamine development. As can be seen from Table 2.2, several Cameroon government officials and scientists, including Mr. Edou, visited the NCI under the sponsorship of NCI. This collaborative mechanism with the Cameroon government was essential to maintaining progress

since, at the time, few legal regulations were in place addressing the study of Cameroonian plants for non-timber uses [74].

2.4 Marine Sources

Marine organisms are proving to be a valuable source of potential anticancer agents, and interested readers are referred to several reviews for details [75, 76]. In the context of drug discovery from African marine resources, it is interesting to note that the first collections of *Dollabella auricularia*, source of the dolastatins [77], were made by Professor G. R. Pettit of Arizona State University off the coast of Mauritius in the early 1960s. Likewise, the first member of the hemiasterlin family of compounds was isolated by Professor Yoel Kashman of Tel Aviv University from the sponge, *Hemiasterella minor* (Kirkpatrick), collected in Sodwana Bay, South Africa [78]. Both the dolastatins and the hemiasterlins have given rise to anticancer agents which have progressed to clinical trials [77, 78]. Collections of the marine worm, *Cephalodiscus gilchristi*, by the Pettit group off the southeastern shores of South Africa in 1988 yielded cephalostatin 1 [79], and further large-scale collections led to the isolation of a further 18 members of this family. These and the closely related ritterazines have been reviewed [80].

In the past decade, the NCI has performed marine invertebrate collections along the coasts of Eastern and Southern Africa through its contract with Coral Reef Research Foundation (see Sect. 2.1.2). Collections off the Southern African coast have been performed in close collaboration with Professor Michael Davies-Coleman through a Memorandum of Understanding with Rhodes University (Table 2.4). Since aspects of marine bioprospecting in Southern Africa are presented in a later chapter in this volume, no further discussion will be included here.

2.5 Conclusions

From the foregoing discussion, it is clear that natural products have made, and continue to make, an indispensable contribution to the discovery and development of effective drugs for the treatment of cancer. This observation applies equally well to many other diseases afflicting humankind. A recent analysis of the new drugs marketed over the past 25 years during the period between 1981 and 2006 shows that some 50% owe their origin in one way or another to natural sources, and in some disease areas well over 60% are derived from natural products [81]. In addition, natural products are an invaluable source of molecular probes in the study of pathways influencing cell cycle progression [82].

While natural products are a proven source of novel bioactive molecules, the actual compound isolated from the natural source is often not suitable for

development into an effective drug, but it may be regarded as a lead molecule which can form the basis for further chemical or biochemical modification. The discovery of promising bioactive molecules always involves close collaboration with biologists, firstly in the collection of the source organisms and then in the provision of suitable disease-oriented screens, while the optimization of the lead molecule requires significant input from medicinal and synthetic chemists. The preclinical development of an agent always requires close collaboration with pharmacologists and toxicologists in the determination of the optimal pharmacodynamic and toxicological parameters required for advancement of the agent into clinical trials with human patients. The recent establishment of the Drug Discovery and Development Center under the leadership of Professor Kelly Chibale at the University of Cape Town, South Africa, exemplifies this multidisciplinary collaborative approach and will play a major role in promoting the discovery and development of drug candidates by African scientists for diseases afflicting the continent.

From the NCI experience, African biodiversity has been shown to be the source of several promising anticancer drugs, with some in advanced clinical trials and others in preclinical development. In addition, the discovery of the anti-HIV-active compound michellamine B has emphasized the need for source countries to establish policies for promotion of the exploration of their valuable biological diversity for the discovery and development of non-timber products, including novel bioactive molecules. One of the cornerstones of the NCI drug-discovery program is collaboration with research groups worldwide in the testing of their products, and the development of promising anticancer leads. In the natural products area, the signing of Memoranda of Understanding with qualified research groups is the mechanism most suited for such collaboration, and it is hoped that more such collaborations will be established with African research groups in the years ahead.

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