Anti-Cancer Drug Discovery and Development in Brazil: Targeted Plant Collection as a Rational Strategy to Acquire Candidate Anti-Cancer Compounds

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Key Words. Drug discovery · Natural products · South America · Compound acquisition · Compound selection

ABSTRACT

Throughout medical history, plant products have been shown to be valuable sources of novel anti-cancer drugs. Examples are the *Vinca* alkaloids, the taxanes, and the camptothecins, derived from the Madagascan periwinkle plant *Catharanthus roseus*, the Pacific yew *Taxus brevifolia*, and the Chinese tree *Camptotheca acuminata*, respectively. For this reason, the South-American Office for Anti-Cancer Drug Development has implemented a large-scale project of acquisition and testing of compounds isolated from South American medicinal plants. The species are selected on the basis of a potentially useful phytochemical composition by consulting ethnopharmacological, chemosystemic, and ecological information. The collected samples are dried and first extracted with an organic solvent, then with distilled water. These crude extracts are evaluated at a concentration of 50 µg/ml for antiproliferative activity against one cell line. Extracts that significantly inhibit the growth of the cells (≥50%) at relatively low concentrations (≤50 µg/ml) are submitted to the more comprehensive disease-oriented screen of the U.S. National Cancer Institute. In parallel, these samples are further purified by bioassay-guided purification, involving repeated fractionation by diverse chromatography methods. If the active substance is expected to represent a novel structure, it is identified by appropriate chemical techniques, mechanistic studies are performed with a wide diversity of tumor models and laboratory techniques, and efforts are undertaken for the synthesis of potentially more useful analogs. The Oncologist 2000;5:185-198

BACKGROUND

Approximately five decades of systemic drug discovery and development have established a respectable armamentarium of useful chemotherapeutic agents [1, 2], as well as a number of important successes in the treatment and management of human cancer [3]. Nevertheless, the need for more effective antineoplastic agents remains. The most common tumors of the adult are resistant to available antineoplastic drugs [4, 5], and the majority of these agents have only limited anti-solid tumor activity [1, 2]. Table 1 illustrates the latter statement, showing that only 5 of the 25 listed commonly used anti-cancer drugs elicit preferential anti-solid tumor activity. Furthermore, these agents have little impact on survival rates [4]. This problem has recently been addressed in a contribution from medical oncologists from five continents, all arriving at the same conclusion on the inadequacy of current chemotherapeutic agents for the treatment of advanced solid malignancies [6].

These considerations led to critical cost-versus-benefit assessments of traditional drug discovery methodologies and to many attempts to improve their efficiency. The currently dominating strategy involves the use of high-throughput chemistry and high-throughput screening [7], which allows for the rapid production and evaluation of large numbers of candidate compounds. Thus, using robust combinatorial chemical approaches, large numbers of structurally related compounds are produced [8, 9], which are subsequently entered into high-throughput screens. In this way, entire libraries of potentially bioactive synthetic...
peptides, oligonucleotides, and small organic molecules have been synthesized and evaluated [10].

The results obtained thus far did not fulfill expectations, principally because most of the peptides and oligonucleotides did not have useful pharmacological properties [10], while the chemical diversity of the more “drug-like” small organic compounds was substantially less than was hoped [10].

These limitations led to the reappraisal of another major source of chemical diversity that has consistently proven its value for the development of novel drugs: natural products [11-13]. This switch in acquisition policy stemmed from the realization that nature, even when compared with the powerful combinatorial chemistry techniques, provides candidate compounds which have more “drug-like” properties (i.e., in terms of absorption and metabolism) as well as a greater chemical diversity (i.e., to allow for structure-activity studies) [14]. Once acquired, these substances can be entered into high-throughput screens, and the lead compounds that emerge can be optimized by combinatorial chemistry, or, in economically less privileged countries, by traditional clinical chemistry approaches.

Admittedly, drug development strategies based on naturally derived candidate compounds may present a considerable number of obstacles which are not posed by those using rational synthesis. There may be problems of procurement due to inaccessibility of collection sites, difficulties with the isolation and production of the pharmacologically active ingredient, and serious legal disputes among governments about intellectual rights properties. Even so, screening of natural products seems more likely to yield a hit when compared with screening of rationally designed compounds.

The appreciation of the significance of natural products as sources for structurally novel and mechanistically unique drugs and the enormous biodiversity of the South American forests were also at the basis of the recent initiative of the South American Office for Anti-Cancer Drug Development (SOAD) to implement a large-scale program of collection and anti-cancer testing of South American plant species. The SOAD is located in Porto Alegre, Brazil, and operates in close collaboration with the U.S. National Cancer Institute (NCI), the European Organization for Research and Treatment of Cancer (EORTC), the British Cancer Research Campaign (CRC), the pharmaceutical industry, and individual research laboratories. This paper describes some of the SOAD’s drug discovery activities.

**The Impact of Naturally Derived Agents on Human Therapeutics**

The widely held perception of nature as a collection of animals moving against a green background of plants is a serious misunderstanding of the complexity and dynamics of the earth’s vegetation. Indeed, plants perform all the living functions which the layman would superficially ascribe only to animals, including communication and defense. For these purposes, they make use of sophisticated signaling mechanisms such as pheromones, and an elaborate chemical arsenal of deadly weapons such as terpenes to poison the soil to inhibit competitors, and alkaloids which make them unpalatable to insects and predators.

For instance, certain trees produce large quantities of tannins in their leaves which are poisonous to animals. If a predator begins to eat one tree in a grove, that tree releases ethylene into the air, which causes other trees in the grove to increase the production of leaf tannin. Within a few minutes, the other trees are producing more tannin, making themselves poisonous. Other plants produce tannin and phenol as a defense against caterpillars. A whole grove of trees is alerted as soon as one tree is infested. Still others produce an

| Table 1. Relative antisolid tumor activity of commonly used antineoplastic drugs |
|----------------|----------------|----------------|
| Exclusively used against hematological malignancies | Used against hematological and solid malignancies | Exclusively used against solid malignancies |
| L-asparaginase | Bleomycin | Carboplatin |
| Busulfan | Cyclophosphamide | Cisplatin |
| Chlorambucil | Daucarbazin | 5-Fluorouracil |
| Cytarabine | Dactinomycin | Mitomycin C |
| Daunorubicin | Doxorubicin | Tamoxifen |
| Mechloretamine | Etoposide | 6-Mercaptopurine |
| 6-Mercaptopurine | Ifofamide | Procarbazine |
| Procarbazine | Melphalan | 6-Thioguanine |
| 6-Thioguanine | Methotrexate | Vinblastine |
anti-feedant chemical when attacked by beetles—and so do other plants in distant parts of the forest. This happens in response to a warning allelochemical secreted by the trees under attack.

Human beings have used some plant constituents for centuries, e.g., to prepare poisonous spearheads for warfare and hunting. In addition, plant-derived substances have traditionally played important roles in the treatment of human diseases. Today, about 80% of the world population residing in third world countries still rely almost entirely on plant products for their primary health care. The remaining 20% of individuals living in the first world use, in more than 25% of cases, pharmaceuticals which have been directly derived from plant products [15, 16]. These range from common remedies such as aspirin (originally isolated from the Rosacea Filipendula ulmaria), to prescription drugs such as the analgesic morphine and the cardiac glycoside digoxin (isolated from the Papaveracea Papaver somniferum, and the Apocynacea Digitalis purpurea, respectively). Table 2 shows a number of well-known drugs that are directly developed from plant species. A more complete list is presented in [17] and [18].

Plant-derived compounds were also of great significance to cancer therapy (Table 3). It was, for instance, only upon the addition of the Vinca alkaloid vincristine or oncovin (isolated from Catharanthus roseus, Apocynaceae [19]) to mechlorethamine, prednisone, and procarbazine (the MOPP regimen) that the first cures in a human cancer (Hodgkin’s disease) were achieved [20]. The combination of the epipodophyllotoxin etoposide (derived from the mandrake plant Podophyllum peltatum and the wild chervil P. emodi, Berberidaceae [21]), bleomycin, and cisplatin is currently a highly active and curative regimen in testicular cancer [22]. Etoposide is furthermore one of the most active agents against small cell lung carcinoma [1, 2, 14, 22].

The more recent development of the structurally and mechanistically novel taxanes (extracted from the bark of the Taxaceae Taxus brevifolia, T. canadensis, or T. baccata [23]) and the camptothecins (derived from the bark and wood of the Nyssaceae Camptotheca acuminata [24]) in the 1990s represented a landmark in cancer research because of their significant anti-solid tumor efficacy. Paclitaxel is in many countries approved for the treatment of ovarian and breast carcinoma and also has important activity against non-small cell lung cancer [25]. Irinotecan and topotecan are semi-synthetics from the lead compound camptothecin which are approved for the treatment of advanced colorectal cancer [26], and as second-line chemotherapy in ovarian carcinoma [27], respectively. These agents are also active against several other solid malignancies such as carcinoma of the lung, cervix, and ovary [26, 27].

Table 4 shows a number of plant-derived agents that are now in experimental use. Homoharringtonine, for instance, is an alkaloid isolated from the Chinese tree Cephalotaxus harringtonia (Cephalotaxacea) [28] and has shown efficacy against various leukemias [29]. 4-Ipomeanol is a pneumotoxic furan derivative isolated from the sweet potato Ipomoea batatas (Convolvulaceae) [30] that has

<table>
<thead>
<tr>
<th>Drug</th>
<th>Medical use</th>
<th>Plant source</th>
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<tbody>
<tr>
<td>Aspirin</td>
<td>Analgesic, anti-inflammatory</td>
<td>Filipendula ulmaria</td>
</tr>
<tr>
<td>Atrpine</td>
<td>Pupil dilator</td>
<td>Atropa belladonna</td>
</tr>
<tr>
<td>Benzoin</td>
<td>Oral disinfectant</td>
<td>Styrax tonkinensis</td>
</tr>
<tr>
<td>Caffeine</td>
<td>Stimulant</td>
<td>Camellia sinensis</td>
</tr>
<tr>
<td>Codeine</td>
<td>Analgesic, antitussive</td>
<td>Papaver somniferum</td>
</tr>
<tr>
<td>Digoxin</td>
<td>For atrial fibrillation</td>
<td>Digitalis purpura</td>
</tr>
<tr>
<td>Eugenol</td>
<td>For toothache</td>
<td>Scyzgium aromaticum</td>
</tr>
<tr>
<td>Hyoscymamine</td>
<td>Anticholinergic</td>
<td>Hyoscyamus niger</td>
</tr>
<tr>
<td>Morphine</td>
<td>Analgesic</td>
<td>Papaver somniferum</td>
</tr>
<tr>
<td>Papaverine</td>
<td>Antispasmodic</td>
<td>Papaver somniferum</td>
</tr>
<tr>
<td>Pilocarpline</td>
<td>For glaucoma</td>
<td>Pilocarpus jaborandi</td>
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<tr>
<td>Quinine</td>
<td>For malaria prophylaxis</td>
<td>Cinchona pubescens</td>
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<tr>
<td>Reserpine</td>
<td>Antihypertensive</td>
<td>Rauwolfia serpentina</td>
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<tr>
<td>Scopolamine</td>
<td>For motion sickness</td>
<td>Datura stramonium</td>
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<tr>
<td>Toxiferine</td>
<td>Relaxant in surgery</td>
<td>Strychnos guianensis</td>
</tr>
<tr>
<td>Xanthotoxin</td>
<td>For vitiligo</td>
<td>Ammi majus</td>
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Table 2. Nontoxic drugs developed from plant sources

<table>
<thead>
<tr>
<th>Drug</th>
<th>Mechanism of action</th>
<th>Plant source</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vinblastine, vincristine</td>
<td>Inhibition of tubulin polymerization</td>
<td>Catharanthus roseus (Apocynaceae)</td>
</tr>
<tr>
<td>Etoposide, teniposide</td>
<td>Inhibition of topoisomerase II</td>
<td>Podophyllum peltatum, P. emodi (Berberidaceae)</td>
</tr>
<tr>
<td>Paclitaxel, docetaxel</td>
<td>Promotion of tubulin stabilization</td>
<td>Taxus brevifolia (Taxaceae)</td>
</tr>
<tr>
<td>Irinotecan, topotecan, 9-aminocamptothecin, 9-nitrocamptothecin</td>
<td>Inhibition of topoisomerase I</td>
<td>Camptotheca acuminata (Nyssaceae)</td>
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been under clinical evaluation as a lung-cancer-specific antineoplastic agent [31]. Elliptinium is a semi-synthetic derivative from ellipticine, which can be derived from Apocynaceae such as Bleekeria vitensis [32] and which is presently used in Europe in the treatment of advanced breast cancer [33].

Flavopiridol is among the most exciting plant-based agents that are currently in development, representing the first anti-cancer agent that targets cell cycle progression. Flavopiridol is a synthetic flavone derived from the plant alkaloid rohitukine, which was isolated from the leaves and stems of Amoora rohituka and later from Dysoxylum binec- tariferum (Maliaceae) [34, 35]. The mechanism of action of flavopiridol involves interference with the phosphorylation of cyclin-dependent kinases, hampering their activation, thus blocking cell cycle progression at G\(_1\) or G\(_2\) [36, 37].

In phase I clinical trials with flavopiridol [38, 39], secretory diarrhea was found to be the dose-limiting toxicity, and encouraging response rates were noted in a variety of solid and hematological malignancies. These results led to the initiation of phase II trials in patients with colorectal, prostate, non-small cell lung, and renal cell carcinoma, as well as non-Hodgkin’s lymphoma and chronic lymphocytic leukemia. Based on in vitro synergy of flavopiridol with several conventional cytotoxic agents [40], phase I combination studies to evaluate flavopiridol plus paclitaxel or cisplatin against advanced solid tumors are also ongoing.

### Targeted Acquisition of Candidate Compounds: The SOAD Approach

As already mentioned, tropical rain forests, with their extremely rich vegetation, offer great possibilities for the discovery of promising new drugs. The Amazon, for instance, covers about 2,722,000 square miles of the earth’s surface and harbors approximately one-third of the global biodiversity [41]. This means an estimated 90,000 higher species, only about 10% of which have been taxonomically characterized [41]. On the other hand, the U.S. and Canada together contain fewer than 2,000 higher plant species [41], among which, nevertheless, the paclitaxel-producing Taxus brevifolia [23] was identified. Furthermore, in a period of five years, the isolation of some 250 new alkaloids from Amazonian plant species has been reported [41, 42]. Since alkaloids represent only one type of the numerous secondary organic compounds in plants, one can easily imagine the chemical wealth that lies awaiting in this vegetal pharmacy [43, 44].

Many large-scale operating governmental research institutions and industrial pharmaceutical laboratories in the more developed countries often employ random acquisition and screening, applying, for instance, the high-throughput approaches mentioned above. Such strategies have been estimated to require the evaluation of approximately 20,000 candidate compounds to obtain one clinically useful drug [43, 44]. By employing a strategy of purposeful, targeted acquisition of preselected plant species, the SOAD hopes to avoid the collection of species already evaluated, instead concentrating on poorly characterized samples with potentially useful phytochemical properties. To this end, we make use of ethnopharmacological, chemosystemic, and ecological information as outlined below.

#### Compound Acquisition Through Ethnopharmacology

Ethnopharmacological data are obtained by consulting traditional healers and by accumulating information on the popular medicinal use of plants, but also from literature on folk medicine. As an example, anecdotal information on the treatment of swellings and warts with certain herbs may provide useful clues about the species upon which to focus during plant collections. In addition, a compilation of more than 3,000 reports on the folkloristic use of plants for treating “cancer” [45, 46], and a number of excellent reviews on plant species having anti-cancer properties [47-50], assist in initially selecting potentially suitable plant families for collection.

The epipodophyllotoxins and the taxanes exemplify that an ethnopharmacological approach for the acquisition

<table>
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<th>Table 4. Some cytotoxic drugs developed from plant sources</th>
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<tr>
<td><strong>Drug</strong></td>
</tr>
<tr>
<td>Homoharringtonine</td>
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<td>4-Ipomeanol</td>
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<tr>
<td>Elliptinium</td>
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<td>Flavopiridol</td>
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of candidate compounds may pay off. Extracts from the rhizomes of Podophyllum emodi and P. peltatum and from the bark of Taxus brevifolia and T. canadensis have for centuries been used against “cancer” by the natives of Asia and North America, respectively [51-53]. Similarly, the popular use in Brazil of, for instance, an alcoholic extract from Maytenus ilicifolia (Celastraceae) called “cancorosa,” or an aqueous extract from Aloe vera or A. arborescens (Liliaceae) called “babosa” [54, 55], represented a lead to evaluate these as well as related species for potential antineoplastic activity. These activities may be attributable to the presence of tubulin-interfering maytansinoids [56, 57], or certain immunomodulating substances [58], respectively, encountered in certain members of these genera.

Popular use in South Brazil with “dragon’s blood,” the red latex from Christ’s crown Euphorbia milli (Euphorbiaceae) [45, 46, 54, 55] as a treatment for warts, also represented a valid clue to look more closely at genera from this plant family. One only has to think of the experience with podophyllotoxin, the alcoholic extract of the roots of Podophyllum plants. This compound became the lead for etopoic and teniposide [21] after years of successful use against warts associated with the venereal disease condylomata acuminata [59]. Interestingly, many members of the Euphorbiaceae contain potent molluscidial lectins and/or diterpenes, which have been considered for snail control to reduce the prevalence of schistosomiasis in endemic areas of South America and Africa [60, 61]. Of note, some of these compounds are also widely used in Chinese folk medicine for the treatment of “cancer” [48, 62], and appear to indeed have potent in vitro cytotoxicity [62, 63].

The folkloristic use of certain plants as an abortive in Northeast Brazil represented another lead to collect and test these and related species. Such an apparent teratogenic effect signified antiproliferative activity, and thus potential antineoplastic activity. Examples of plants having such a traditional use are some species from the genera Ruta (Rutaceae) and Casearia (Flacourtiaceae) [45-47]. Indeed, certain Ruta and Casearia contain potent carcinogenic diterpenes [64-67], and the Rutaceae genus Zanthoxylum contains benzophenanthridine alkaloids with a variety of biological activities, including interference with topoisomerase I [68, 69], protein kinase C [70], and tubulin metabolism [71].

Because in many cases the composition of the pharmacologically active substances of ethnomedicinal compounds is not known or is being held secret by the traditional healers, it is generally agreed that compound acquisition on the basis of this criterion is likely to yield entirely novel antineoplastic drugs.

**Compound Acquisition Through Chemosystemics**

Chemosystemics involves the use of knowledge about the phytochemical composition of a certain species, genus, or family as a clue to evaluate related species for the presence of structurally comparable substances with an improved therapeutic index. Thus, this strategy is likely to result in the identification of new analogs rather than novel lead compounds.

A recent example of an anti-cancer drug that emerged through this approach is docetaxel [72]. This agent was developed by hemisynthesis from baccatin, an alkaloid isolated from the needles of the European yew Taxus baccata, in order to meet the initial developmental problems with paclitaxel isolated from the bark of the American yew T. brevifolia [23].

Similar considerations lead us to collect species from a variety of genera such as Physalis (Solanaeaceae) and Combretum (Combretaceae). Extracts from these plants may contain clinically more useful analogs of physalins or combretastatins, respectively [46-49]. Physalins are lactones present in the ethanolic extract of Physalis species, which inhibited the growth of several human leukemia- and solid tumor-derived cell lines in vitro and/or in vivo [73-75]. Combretastatins are stilbene derivatives that may be useful as anti-tumor agents due to their capacity to inhibit tubulin polymerization [76-79] and to suppress angiogenesis by inducing vascular shutdown [80].

**Compound Acquisition Through Ecological Survey/Rational Search**

Such a strategy for compound acquisition is expected to yield both new leads and analogs and can be best explained by a practical example. The presence of a single plant species free of fungi in a large area of dense vegetation almost completely covered by fungi, signified that such a species might produce a fungicidal, potentially cytotoxic substance worthy of testing for its antiproliferative potential. Thus, this approach for compound acquisition exploits the diversity of chemical substances produced by plants in response to environmental stress.

On the basis of this reasoning, we collected and processed Quillaja species (Rosaceae) and several Asteraceae and Leguminosae. Some Quillaja members produce saponins, supposedly as a defense mechanism against attack by insects [81]. Some of these substances displayed adjuvant activity in vaccination protocols, conferred protection against certain infectious diseases, and boosted the humoral immune response [82, 83].

Regarding the plant families Asteraceae, Composites, or Leguminosae, many of their members produce pyrrolizidine alkaloids—potent cytotoxic, carcinogenic, and/or anti-carcinogenic compounds [84, 85]—which probably act by crosslinking DNA [86]. One of these compounds, indicine N-oxide, advanced to clinical trial [87, 88], but unremarkable
anti-tumor effects and unacceptably severe, irreversible hepatotoxicity led to the termination of its further development.

Plant Collections

Employing the above-mentioned criteria to select certain plants for collection, our botanists first consult herbaria and relevant literature to establish whether the desired species, genera, or families can be encountered in South America. If so, for each species, a diagram is made that marks the locations where that species is likely to be found. Also, for each species, the ideal season for collection is verified. Using these "maps" (Fig. 1), a certain area is designated for

Figure 1. Maps indicating the distribution of Hypericum species in the South-Brazilian state of Rio Grande do Sul. A) H. brasiliensis var. brasiliense; B) H. brasiliense var. linoides; C) H. caprifoliatum; D) H. connatum; E) H. cordiforme; F) H. denudatum.
collection. For reasons of logistics, plants are now principally collected in the South Brazilian states of Rio Grande do Sul and Santa Catarina, as well as in the Atlantic and Amazon forests. Figures 2 and 3 show two typical collection sites in the Amazon.

Before organizing expeditions, permission must be obtained from the Brazilian government. This is to limit the number of collections per area, to prevent excessive collection of rare species, and to provide guidelines for responsible expeditions. Thus, a particular location is visited only twice per year, protected species are not collected, no trees are felled, samples of bark are taken from only one side so as not to gird the tree, and root samples are taken only from the periphery.

If possible, several parts of a plant are collected (leaves, twigs, bark, flowers, fruits, and/or roots), which are separately cataloged. In general, 50 to 100 grams of a sample must remain after drying in order to allow for the preparation of sufficient extract to carry out the initial tests. Depending on the succulence, this may require the collection of as much as 2 kg of leaves or 500 grams of roots and twigs. Although the aim is to collect a wide variety of plants, certain species possessing insufficient biomass may pose difficulties.

Therefore, we concentrate on shrubs, trees, and bushes. Samples are labeled in the field and returned to the base camp for several days of drying. There, they are weighed, taxonomically characterized, placed in boxes, and shipped to our extraction laboratory.

The shipments are accompanied by the complete taxonomy of the species collected, as well as the plant parts collected, location and date of collection, rationale for collection, etc. Also, voucher specimens are prepared (Fig. 4) and are deposited at several university herbaria for future reference.

**Extract Preparation and Testing**

The dried plant material is macerated and first eluted with an apolar solvent such as chloroform/methanol to remove the lipophilic components, then with distilled water to obtain the hydrophilic constituents. The organic and aqueous extracts are concentrated by rotary evaporation, or lyophilization, respectively, divided in aliquots of 25 mg, labeled, and stored at -20°C until testing.
These crude extracts, as well as (partially) purified samples prepared from them, are in the first instance evaluated for their cell growth-inhibiting capacity at only one concentration, and against only one cell line. This decision is based on the realization that in these initial stages only the degree of cell growth inhibition is necessary to judge whether to discard the sample or to continue with its purification. The cell line used is selected on the basis of relevant biological information such as growth rate, known characteristics of cellular targets, and tumor origin, as well as preliminary knowledge about chemical composition and/or mechanism of action of the allegedly pharmacologically active ingredient in the test sample. In order to reduce the number of false-positive results, experiments are performed under conservative conditions, taking as the criterion for “activity” the ability of the sample to inhibit cell growth by at least 50% at the relatively low single dose of 50 µg/mL.

Thus, cancer cells are inoculated into the wells of microtiter plates and incubated for three days in the absence or presence of the extract. At the end of the incubation period, cells are fixed in situ, stained with sulforhodamine B (a dye that stains the basic amino acids of proteins), and assessed by means of a colorimeter for their cell growth-inhibiting capacity [89].

Samples that pass the preliminary screen are further evaluated in the 60-human cell line panel of the NCI [89-92]. This panel incorporates nine tumor histotypes, viz., leukemia, malignant melanoma, and glioma, as well as breast, lung, ovarian, kidney, colon, and prostate carcinoma-derived cell lines. Anti-tumor activity is judged as the ability of the test compound to selectively kill or inhibit the growth of certain cell lines, rather than general cytotoxicity against all or most cell lines of the panel. Thus, a substance that displays unusual, selective patterns of cytostatic or cytotoxic activity advances to further evaluation.

If warranted by the combined data from our tests or those of the NCI, the brutus extracts are further fractionated. These fractions are also evaporated to dryness and retested, again at 50 µg/mL and for three days, against the previously used cell line. Fractions that inhibit cell growth at least at levels similar to those attained with the crude extract are selected for further purification by, for example, preparative chromatography techniques. The subfractions obtained are again evaluated as mentioned above. This process of bioassay-guided purification is continued until a sufficiently pure substance is obtained to allow for structure identification by procedures such as nuclear magnetic resonance spectroscopy.

If warranted by structural novelty, the purified compound is evaluated in animal models at institutions such as the EORTC and/or the CRC. Furthermore, a battery of in vitro studies is performed using a wide diversity of tumor models and laboratory techniques in order to more definitely establish its mechanism of action. Also, efforts are undertaken for the synthesis of potentially more useful analogs.

**Current Research Lines**

Thus far, about 1,500 extracts have been prepared from about 500 species, which are in various stages of our preclinical drug discovery program. Some of these developments are addressed hereunder, and Table 5 summarizes a few other details of the SOAD program.

**Maytenus**

The popular use of “cancorosa” has its scientific rationale due to the presence in *Maytenus* plant species of ansa macrolides with known cytotoxic effects [56]. The potent tubulin inhibitor maytansine, for instance, was first isolated from the African *Maytenus serrata* in the 1960s [101] and was particularly effective in preclinical leukemia models [57]. However, in subsequent early clinical studies, maytansine was found to produce severe toxicity and unimpressive anti-tumor effects [57], which lead to the termination of its further development.

Notwithstanding, a durable partial response was documented in a patient with islet cell carcinoma, a characteristically chemotherapy-resistant tumor [102]. Also, in a comparison of tubulin-interfering agents for their performance in the NCI’s 60-human cell line panel, maytansine ranked second only to paclitaxel [103], confirming its considerable anti-tumor potential. Moreover, more recently, a number of strongly cytotoxic novel maytansinoids and aromatic triterpenes have been identified in *M. emarginata* [104], and *M. ilicifolia* and *M. chuchuhuasca* [105], respectively.

For these reasons, we are currently evaluating extracts from the six *Maytenus* species indigenous to the South Brazilian state of Rio Grande do Sul for their antineoplastic potential. These include *M. aquifolia*, *M. boaria*, *M. cassineformis*, *M. dasyclada*, *M. ilicifolia*, and *M. robusta*. Testing against the U-373 human glioma cell line (unpublished observations) showed a potent antiproliferative effect (IC₅₀ value of 5-10 µg/mL) of the organic, but not the aqueous extracts prepared from the leaves and the twigs of these plants. Neither extract from the other plant parts exerted appreciable growth-inhibitory effects. Initial testing of the active fractions in an in vitro assay for tubulin inhibition [106] gave positive results, hinting toward the possible presence of maytansinoids. Bioassay-guided purification of these samples is ongoing.

**Tabebuia**

Lapachol, a naphtalenedione first isolated from the wood of *Stereospermum suaveolens* (Bignoniaceae), was...
found to inhibit the growth of the Walker 256 rat carcinoma cell line when given orally [107]. Mostly on the basis of this observation, lapachol was carried into clinical trial. Despite the lack of significant toxicity even at large oral doses, sufficiently high blood levels were not attained to show a therapeutic effect [108]. This led to the termination of the further clinical development of lapachol.

Naphtalenediones are also found in the heartwood of Asian and South American Bignoniaceae such as species from the genera Tabebuia, Taigu, and Tecoma (e.g., Surinam greenheart). Interestingly, extracts from these plant parts have long been popularly used in Brazil for “cancer,” while a Jacaranda species called “cancer bush” is popularly used in the Bahamas for skin cancer [54, 55].

Of note, the naturally occurring lapachol analog β-lapachone has been shown to be an inhibitor of DNA topoisomerase I, with a mode of action different from camptothecin and a chemical structure distinct from that of current anti-cancer drugs [109]. This compound induced cell death, furthermore, with characteristics of apoptosis in human prostate cancer cell lines at concentrations of less than 8 µM [109]. Interestingly, this effect was independent of p53 expression, and ectopic overexpression of bcl-2 did not confer significant resistance to β-lapachone [109].

These data led us to submit partially purified samples derived from the wood of a Tabebuia species for testing in the NCI’s 60-cell line panel, after a crude organic extract displayed significant cell growth inhibition in our prescreen. In the NCI’s screen, this sample displayed preferential selective cytotoxicity against the renal carcinoma subpanel [110, 111]. Subsequent bioassay-directed purification in our laboratory suggested that the active component was a lapachol-like naphtoquinone [110, 111]. At this moment, preparations are being made for larger-scale collection in order to carry out further fractionation and chemical identification studies.

**Hypericum**

Our enthusiasm for this plant genus (family Guttiferae) was sparked by the protein kinase C-inhibiting properties of the hypericinoids present in the North American St. John’s Wort *Hypericum perforatum* [112]. Abnormalities in one or more aspects of protein kinase C have been associated with various disorders of the central nervous system, ranging from degenerative diseases to brain malignancies [113]. Hypericinoids may therefore not only be useful against mental ailments—crude extracts of *H. perforatum* have for years been sold as “over-the-counter remedies” against depression [114]—but also in the therapy of malignant gliomas.

Indications for the latter suggestion were provided by the significant preclinical anti-glioma activity of these substances [115]. Importantly, a patient with glioblastoma multiforme who was refractory to tamoxifen—also an inhibitor of protein kinase C that may elicit an anti-glioma effect—responded to subsequent therapy with hypericin [116].

Hypericinoids have further been shown to possess anti-retroviral activity in vitro and in vivo [117], suggesting their therapeutic use against, for example, AIDS. The results of a study with anti-mutagens from plant extracts having potential modulating effects on DNA repair in *Escherichia coli* bacteria indicated a further antimutagenic effect of *H. perforatum* that was probably due to the suppression of error-prone repair [118]. In addition to hypericinoids, plant species from the genus *Hypericum* may contain various other bioactive products including xanthones, benzopyranes, as well as phloroglucinol and filicinic acid derivatives. The phloroglucinol derivative hyperforin, for example, has also been found to have antiproliferative properties [119].

With this background, we decided to evaluate (partially) purified extracts from seven South-Brazilian *Hypericum* species for their antiproliferative effects in vitro. We also examined whether one or more of the above-mentioned growth-inhibiting compounds were present in the samples, and whether inhibition of protein kinase C was indeed related to their antiproliferative properties. The species evaluated included *H. caprifoliatum, H. carinatum, H. conantum, H. cordatum, H. myrianthum, H. piriai*, and *H. polyanthemum* (Fig. 5). Voucher specimens have been prepared and deposited at the Herbarium of the Federal University of Rio Grande do Sul (ICN). The data obtained up to now have been presented in abstract form [120-122].

The aerial parts of the plants were air-dried, powdered, and fractionated by successive extraction with petroleum ether, petroleum ether:chloroform (1:1), chloroform, and methanol. The samples were evaporated to dryness, and tests against the A-172 human glioma cell line localized the greatest growth-inhibiting activity to the petroleum ether fractions (IC₅₀ values 30-40 µg/ml). Repeated preparative thin-layer chromatography was used to clean up these fractions, whereby activity against the A-172 cell line was used to select the subfractions for further purification. Thus, subfractions producing at least 50% inhibition of the growth of these cells at concentrations ≤50 µg/ml were further purified. The progress of the purification process was monitored by analytical high-performance liquid chromatography. Using commercially available standards, this technique was also used to exclude the presence of hyperforin, hypericin, and/or pseudohypericin in the active samples.

The sufficiently purified active samples were chemically characterized by nuclear magnetic resonance spectroscopy, which revealed the presence of novel benzopyranes (Fig. 6), as well as dimers of phloroglucinols and philicinic acid (Fig. 7). In subsequent studies, these compounds were found to inhibit the growth of the A-172 cell line at IC₅₀ values <10...
μg/ml, and to induce at these concentrations characteristics of apoptosis. A histone phosphorylation assay revealed no significant inhibition of protein kinase C activity at the 50% growth-inhibiting concentrations. Currently, these products are undergoing a series of tests in order to establish their mechanism(s) of action.

**CONCLUDING REMARKS**

Although plants have been used for over 3,500 years in the treatment of “cancer,” it was only since the late 1950s that the evaluation of crude plant extracts for their antiproliferative

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**Table 5. Rationale for collecting species in various stages of the SOAD drug discovery program**

<table>
<thead>
<tr>
<th>Species</th>
<th>Family</th>
<th>Ethnopharmacology</th>
<th>Chemosystemics</th>
<th>Ecological survey</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aloe</td>
<td>Liliaceae</td>
<td>Folk use against “cancer” [54, 55]</td>
<td>Immunomodulation [58]</td>
<td></td>
</tr>
<tr>
<td>Bacharis</td>
<td>Asteraceae</td>
<td></td>
<td>In vitro cytotoxicity [93, 94]</td>
<td></td>
</tr>
<tr>
<td>Brucea</td>
<td>Simaroubaceae</td>
<td></td>
<td>In vitro cytotoxicity [95, 96]</td>
<td></td>
</tr>
<tr>
<td>Casearia</td>
<td>Flacourticaceae</td>
<td>Teratogenic [66, 67]</td>
<td>In vitro cytotoxicity [66]</td>
<td></td>
</tr>
<tr>
<td>Combretum</td>
<td>Combretaceae</td>
<td></td>
<td>In vivo and in vitro cytotoxicity [76-79]; angiosuppression [80]</td>
<td></td>
</tr>
<tr>
<td>Eugenia</td>
<td>Myrtaceae</td>
<td></td>
<td>In vitro cytotoxicity [97, 98]</td>
<td></td>
</tr>
<tr>
<td>Physalis</td>
<td>Solanaceae</td>
<td></td>
<td>In vitro cytotoxicity [73-75]</td>
<td></td>
</tr>
<tr>
<td>Quillaja</td>
<td>Asteraceae</td>
<td></td>
<td>Immunomodulation [82, 83]</td>
<td></td>
</tr>
<tr>
<td>Ruta</td>
<td>Rutaceae</td>
<td>Abortive [54, 55]</td>
<td>Frame-shift mutagen [64, 65]</td>
<td></td>
</tr>
<tr>
<td>Zanthoxylum</td>
<td></td>
<td></td>
<td>In vitro cytotoxicity [68-71]</td>
<td>Against pathogens [99, 100]</td>
</tr>
</tbody>
</table>
potential was initiated in earnest. Since then, more than 120,000 plant extracts from over 6,000 genera have been tested, resulting in the development of a large number of widely structurally divergent “natural products” as candidate anti-cancer agents.

Some of these proved to be clinically useful, and others served as tools to unravel the biochemical mechanisms involved in the growth and regulation of tumors. In the latter cases, a broad arsenal of mechanisms of action has been identified. The fact that thus far only a relative handful of natural products have been evaluated for their anti-cancer potential holds promise for the identification of agents acting through even more sophisticated mechanisms.

**REFERENCES**


24. McGuire WP, Hoskins WJ, Brady MF et al. Cyclophosphamide and cisplatin compared with paclitaxel and cisplatin...


30 Rehm S, Devor DE. Acute effects of 4-ipomeanol on experimental lung tumors with bronchiolar or alveolar cell features in Syrian hamsters or C3H/HeNCr mice. J Cancer Res Oncol 1993;120:41-50.


60 Schall VT, Vasconcellos MC, Valent GU et al. Evaluation of the genotoxic activity and acute toxicity of Euphorbia


