Published in final edited form as:

Phytochem Rev. 2014 December ; 13(4): 727–739. doi:10.1007/s11101-014-9335-7.

Investigation of Vietnamese plants for potential anticancer agents

Lynette Bueno Pérez,

College of Pharmacy, The Ohio State University, 500 West 12th Avenue, Columbus, OH 43210, USA

Patrick C. Still,

College of Pharmacy, The Ohio State University, 500 West 12th Avenue, Columbus, OH 43210, USA

C. Benjamin Naman,

College of Pharmacy, The Ohio State University, 500 West 12th Avenue, Columbus, OH 43210, USA

Yulin Ren,

College of Pharmacy, The Ohio State University, 500 West 12th Avenue, Columbus, OH 43210, USA

Li Pan,

College of Pharmacy, The Ohio State University, 500 West 12th Avenue, Columbus, OH 43210, USA

Hee-Byung Chai,

College of Pharmacy, The Ohio State University, 500 West 12th Avenue, Columbus, OH 43210, USA

Esperanza J. Carcache de Blanco,

College of Pharmacy, The Ohio State University, 500 West 12th Avenue, Columbus, OH 43210, USA

Tran Ngoc Ninh,

Institute of Ecology and Biological Resources, Vietnam Academy of Science and Technology, 18 Hoang Quoc Viet, Cau Giay, Hanoi, Vietnam

Bui Van Thanh,

Institute of Ecology and Biological Resources, Vietnam Academy of Science and Technology, 18 Hoang Quoc Viet, Cau Giay, Hanoi, Vietnam

Steven M. Swanson,

College of Pharmacy, University of Illinois at Chicago, 833 South Wood Street, Chicago, IL 60612, USA

Djaja D. Soejarto, and

College of Pharmacy, University of Illinois at Chicago, 833 South Wood Street, Chicago, IL 60612, USA. Department of Botany, Field Museum, 1400 South Lake Shore Drive, Chicago, IL 60605, USA

A. Douglas Kinghorn

College of Pharmacy, The Ohio State University, 500 West 12th Avenue, Columbus, OH 43210, USA

Abstract

Higher plants continue to afford humankind with many new drugs, for a variety of disease types. In this review, recent phytochemical and biological progress is presented for part of a collaborative multi-institutional project directed towards the discovery of new antitumor agents. The specific focus is on bioactive natural products isolated and characterized structurally from tropical plants collected in Vietnam. The plant collection, identification, and processing steps are described, and the natural products isolated from these species are summarized with their biological activities.

Keywords

Anticancer agents; Natural products; Tropical plants; Vietnam

Introduction

Cancer is the second leading cause of mortality in the United States, after heart disease, and is responsible for about one out of every four deaths (American Cancer Society 2013). According to the most recent United States mortality data, 567,628 people died of cancer in 2009, with this being the main cause of death for persons between the ages of 40–79 years (Siegel et al. 2013). It is estimated that in the year 2013, 1,660,290 new cancer cases will be diagnosed and about 580,350 Americans will die of cancer, accounting for approximately 1,600 deaths per day (American Cancer Society 2013; Siegel et al. 2013). Cancer of the lung and bronchus is responsible for the greatest number of deaths, followed by prostate cancer for men or breast cancer for women, and colorectal cancer (American Cancer Society 2013; Siegel et al. 2013). However, cancer death rates have decreased slightly each year by about 1.8% for men and by 1.5% for women, according to the most recent statistics (2005–2009) from the Surveillance, Epidemiology, and End Results (SEER) Program of the U. S. National Cancer Institute (Siegel et al. 2013). The decrease in number of deaths due to cancer has been a result of adopting healthier lifestyles, and from the earlier detection of certain cancers by routine prevention screening, as well as improvements in treatment (American Cancer Society 2013; Siegel et al. 2013).

Natural products play an important role in the treatment of cancer and disease in general. The origin of all approved drugs between the years 1981 to 2010 indicates that 4% are unmodified natural products and 22% are semi-synthetic derivatives (Newman and Cragg 2012). Among cancer chemotherapeutic agents, the proportion of natural product-derived drugs is much more prevalent. According to recent data on approved antitumor agents from

the 1930s to mid-2012, 16% are unmodified natural products, and 31% are semi-synthetic modifications of natural product compounds (Newman and Giddings 2013). Natural product drugs may be sourced equally effectively from fungi, marine organisms, microorganisms, plants, and terrestrial animals (Cragg et al. 2009; Kinghorn et al. 2009; Newman and Giddings 2013; Pan et al. 2012).

The wide use of natural compounds as drugs is based on the structural variety and evolutionary biological roles of compounds isolated from Nature, when compared with synthetic compounds and those produced by combinatorial chemistry (Appendino et al. 2010; Cragg et al. 2009; Feher and Schmidt 2003; Kinghorn et al. 2009; Lachance et al. 2012; Pan et al. 2012). Secondary metabolites are products of enzymatic reactions by an organism or symbiotic consortium, and therefore tend to be intrinsically bioactive and complementary to their target macromolecules. As protein domains are highly conserved, secondary metabolites produced by an organism (e.g., a plant) have been found frequently to be complementary to animal protein targets, explaining the molecular basis for the intrinsic bioactivity of natural compounds (Appendino et al. 2010; Cragg et al. 2009; Kinghorn et al. 2009). Today, synthetic chemists often use lead bioactive natural products as “privileged structures” to build libraries of related compounds for further study (Appendino et al. 2010; Cragg et al. 2009; Feher and Schmidt 2003; Kinghorn et al. 2009; Lachance et al. 2012; Pan et al. 2012). It is estimated that 40% of the pharmacophores found in natural product compounds are absent in synthetic compounds (Appendino et al. 2010). In addition, natural compounds have less rotatable bonds, a greater number of fused rings, bridgehead carbon atoms, and chiral centers, and are more oxygen-rich and contain less nitrogen, sulfur and halogen atoms than synthetic compounds (Appendino et al. 2010; Feher and Schmidt 2003; Kinghorn et al. 2009; Lachance et al. 2012; Pan et al. 2012). Moreover, the chemical space covered by natural compounds is much larger than the chemical space occupied by synthetic compounds, and is very similar to the chemical space of approved drugs (El-Elmat et al. 2012; Feher and Schmidt 2003).

The plant-derived anticancer drugs in the United States used widely in the clinic today can be structurally grouped into four main categories: the camptothecins, [irinotecan (Camptosar®) and topotecan (Hycamtin®)], the epipodophyllotoxins, [etoposide (VePesid®), etoposide phosphate (Etopophos®) and teniposide (Vumon®)], the taxanes, [cabazitaxel (Jevtana®), docetaxel (Taxotere®), paclitaxel (Taxol®), paclitaxel poliglumex (Opaxio®), and albumin-bound paclitaxel (Abraxane®)], and the vinca alkaloids, [vinblastine sulfate (Velban®), vincristine sulfate (Oncovin®), vincristine sulfate liposome (Marqibo®), and vinorelbine tartrate (Navelbine®)] (Bailly 2009; Gordaliza 2007; Kingston et al. 2012; Lucas et al. 2010; Pan et al. 2012; Rahier et al. 2005).

Table 1 presents a comprehensive subset of U.S. FDA new drug application (NDA) approvals from the period January 1, 2011 to June 30, 2013, comprising unmodified natural products isolated from plants, synthetic derivatives of plant natural products, and a refined plant extract marketed as a “botanical drug product”. This table builds on a similar tabulation from a previous review that summarized plant-derived drugs approved during the period 2001 – 2010 (Kinghorn et al. 2011). The “botanical drug product” Fulyzaq® (crofelemer), introduced in 2012, is a mixture of oligomeric proanthocyanidins from *Croton*

lechleri Müll. Arg. (Euphorbiaceae), and used for the treatment of diarrhea associated with HIV/AIDS antiretroviral therapy. As described for Veregen®, the only prior example of a botanical drug product approved by the FDA in 2006, strict quality control, standardization and clinical trial protocols were required prior to NDA approval of Fulyzaq® (Chen et al. 2008). With roughly 500 current investigational new drugs (INDs) or pre-IND applications for botanical drug products now being considered, this appears to be a new drug category with a meaningful future potential (Dou 2013).

Abbreviated NDAs (ANDAs; for generics), applications for INDs (for drugs used in research), and supplemental NDAs (sNDAs; for modifications to approved drugs) were excluded from Table 1, even those sNDAs corresponding to approval of existing drugs for new indications, such as the 2012 supplemental approval of Abraxane® (protein-bound paclitaxel) for therapy of non-small cell lung cancer. Likewise, these same types of NDAs were omitted from Figure 1, which is a graphical illustration of the source of all new drugs approved by the U.S. FDA during calendar year 2012. This shows that, if botanicals are included, 10% of 2012 new drug approvals were produced with minor modification or directly from plants, 27.5% from terrestrial animals, 12.5% from bacteria, 2.5% from marine sources, and 47.5% entirely synthetically. This figure summarizes in a clearly discernible fashion the current net impact of ongoing natural products research on the development of new drugs. This is particularly significant during an era in which natural products research has been discontinued or curtailed by financial cutbacks evident in the pharmaceutical industry, with the void filled by smaller companies, as well as by academic and government laboratories (Kingston 2011).

The remainder of this review summarizes several promising anticancer lead compounds that have been isolated from plants collected in Vietnam as part of the multi-institutional collaborative research falling under the program project P01 CA125066 entitled “Discovery of Anticancer Agents of Diverse Natural Origin”. The funding for this project was provided by the U.S. National Cancer Institute (NCI), National Institutes of Health (NIH), Bethesda, MD. The aim of this research is the discovery of new natural product anticancer lead compounds from tropical plants, filamentous fungi, and aquatic cyanobacteria. The program project integrates the research work of three academic institutions (University of Illinois at Chicago, University of North Carolina at Greensboro, and The Ohio State University), a biotechnology company (Mycosynthetix, Inc., Hillsborough, North Carolina), and a pharmaceutical partner (Bristol-Myers Squibb, Princeton, New Jersey) (Kinghorn et al. 2009; Orjala et al. 2012). Herein, the results are reported of the bioactivity-guided isolation of anticancer lead compounds from Vietnamese tropical plants discovered in our laboratories at The Ohio State University and the University of Illinois at Chicago over the last few years.

Botanical Collections

Altogether, 824 primary plant samples corresponding to 398 species were collected during several plant collection expeditions to Vietnam carried out during the period 2008–2011. Plant specimens were collected as part of a Memorandum of Agreement between the University of Illinois at Chicago and the Institute of Ecology and Biological Resources of

the Vietnam Academy of Science and Technology, Hanoi, Vietnam. Plant parts under evaluation have been the bark, flowers, fruits, leaves, roots, stems, and twigs, either singly or in various combinations. The natural products database, NAPRALERT™ was used for the selection of endemic, phytochemically under-explored plants in Vietnam for study (Loub et al. 1985). The “Indo-Burma” region inclusive of Vietnam was chosen for plant collections since it is considered a “global biodiversity hotspot”, containing about 44% of the world’s plant species but covering only 1.4% of the land area (Malcolm et al. 2006; Rai and Lalramnghinglova 2011). To increase the range of plants collected from Vietnam, expeditions took place in the north mountainous region of Hoanglien National Park, the central region of Kego Nature Reserve Park, as well as further to the south at the Hon Ba Forest Reserve and the Nui Chua National Park. Plant collections and identification were made by two of the authors (T. N. N. and D. D. S.). All plant parts collected were processed at the University of Illinois at Chicago, where these plant samples were dried, milled, packed, and then sent to The Ohio State University for phytochemical and biological studies. Voucher specimens were deposited at the John G. Searle Herbarium of the Field Museum of Natural History at Chicago, Illinois.

Compounds Isolated

From the plants collected in Vietnam and investigated phytochemically thus far, 17 new and 42 known compounds were found to have in vitro activity in one or more bioassays, exhibiting IC₅₀ values of less than 10 µM (Fig. 2 and Table 2). In some cases promising lead compounds have been evaluated against biological targets other than those strictly germane to cancer chemotherapeutic drug discovery. The isolation procedures for the new compounds, perviridisin B (**1**), a cyclopenta[*b*]benzopyran analogue purified from *Aglaia perviridis* and elaeocarpucin C (**32**), a 16,23-epoxycucurbitacin derivative obtained from *Elaeocarpus chinensis*, were directed by LC/MS dereplication and cytotoxicity-guided fractionation using HT-29 human colon cancer cells (Pan et al. 2012; Pan et al. 2013). The structure elucidation of the structurally interesting compound, perviridisin B (**1**), was based on extensive NMR data interpretation. Compound **1** exhibited 11 downfield proton signals in the ¹H-NMR spectrum, characteristic of a cyclopenta[*b*]benzopyran backbone, similar to the values described for previously reported compounds (Inada et al. 2001; Pan et al. 2013). In addition, the ¹H- and ¹³C-NMR spectra showed characteristic signals that suggested the presence of a putresciny 4-hydroxytiglate residue linked by an amide bond to the cyclopenta[*b*]benzopyran skeleton. The C-10 epimer of **1** was also isolated in this study; however, this epimer showed no discernible biological activity (Pan et al. 2013). Potent cytotoxicity against HT-29 cells with IC₅₀ values of less than 1 µM was observed for both perviridisin B (**1**) and elaeocarpucin C (**32**) (Pan et al. 2012; Pan et al. 2013). In addition, compound **1** showed no toxicity to CCD-112CoN normal human colon cells (Pan et al. 2013).

A new caged xanthone, cochinchinoxanthone (**20**), was found to be cytotoxic against the HT-29 cell line and has activity in a mitochondrial transmembrane potential (MTP) assay, with IC₅₀ values of 5.8 and 3.3 µM, respectively (Ren et al. 2011). The structure elucidation of **20** was based on spectroscopic data interpretation and comparison of its NMR spectra to those reported for similar caged xanthones (Thoison et al. 2000; Ren et al. 2010). Also, the

UV ($\lambda_{\max}$ 226, 327, 346 nm), and IR ($\nu_{\max}$ 1744 and 1644 cm^{-1}) data, and three quaternary carbon peaks at δ_{C} 84.0 (C-5), 84.7 (C-13) and 90.3 (C-10_a) ppm, were diagnostic signals used to propose a caged xanthone structure (Ren et al. 2011).

Three new flavanones, (+)-5-methoxypurpurin (**40**), (2*S*)-2,3-dihydrotephroglabrin (**41**), and (2*S*)-2,3-dihydrotephroapollin C (**42**), isolated from *Indigofera spicata*, showed cancer chemopreventive potential by inducing quinone reductase activity, a phase II metabolizing enzyme, in Hepa 1c1c7 murine hepatoma cells (Bueno Pérez et al. 2013). The structures of the new compounds were determined by NMR data interpretation and comparison of their chemical shifts to the values of closely related analogues reported previously in the literature (Abd El-Razek et al. 2007; Rao and Raju 1984; Waterman and Khalid 1980). In addition, the ^1H -NMR data of compounds **40–42** showed typical signals for the presence of a flavanone nucleus with resonances around δ_{H} 5.5 (H-2), 2.9 (H-3_a), and 3.0 (H-3_b) ppm (Bueno Pérez et al. 2013). The absolute configuration assignments were based on circular dichroism and specific rotation measurements. Compounds **40–43** and **48** were active with CD (concentration required to double quinone reductase activity) values of less than 10 μM and low toxicity to cells with IC_{50} values greater than 20 μM (Bueno Pérez et al. 2013). Furthermore, the new flavanone **40** exhibited a low CD value of 0.2 μM and a high IC_{50} and CI (chemopreventive index = IC_{50}/CD) values of 63.9 μM and 376.7, respectively, demonstrating this new compound to be an interesting new lead as a cancer chemopreventive agent (Bueno Pérez et al. 2013). In addition, compound **40** showed more potent induction activity with much lower toxicity to cells than the positive control, L-sulforaphane (CD = 0.5 μM , IC_{50} = 12.1 μM , and CI = 23.9), a well-known monofunctional inducer of quinone reductase obtained from broccoli (Bueno Pérez et al. 2013; Zhang et al. 1992).

Compounds that act upon neural nicotinic acetylcholine receptors (nAChRs) have attracted interest in drug discovery for the potential treatment of autism, cancer, depression and other diseases (Still et al. 2013). A new piperidine alkaloid, microgrewiapine A (**49**), isolated from *Microcos paniculata*, showed cytotoxicity to HT-29 cells with an IC_{50} value of 6.8 μM and also modulated the activity of two nAChRs (Still et al. 2013). The structure determination of **49** was based on extensive spectroscopic data interpretation. The relative configuration of substituents at positions C-2 and C-6 was proposed based on determination of coupling constants and ^1H - ^1H NOESY correlations, whereas the *R*-absolute configuration at the C-3 hydroxy group was assigned using the Mosher ester reaction (Still et al. 2013). Compound **49** exhibited selective cytotoxicity against HT-29 cells when compared to CCD-112CoN normal cells, and also displayed antagonistic activity with 60% and 70% inhibition against the $\text{h}\alpha 4\beta 2$ and $\text{h}\alpha 3\beta 4$ nAChRs, respectively (Still et al. 2013). Microgrewiapine A (**49**) is also the first example of a cytotoxic and nAChR antagonist piperidine alkaloid to have been obtained from a plant in the genus *Microcos* (Still et al. 2013).

The known compounds rocaglaol (**7**), methyl rocaglate (**8**) and five other known rocaglate derivatives (**4–6**, **9–10**), four known cucurbitacins, cucurbitacin D (**36**), 3-*epi*-isocucurbitacin D (**37**), 25-*O*-acetylcucurbitacin F (**38**), cucurbitacin I (**39**), and two known rotenoids, *cis*-(6 $\alpha\beta$,12 $\alpha\beta$)-hydroxyrotenone (**44**) and rotenone (**45**) were also found to exhibit potent cytotoxicity against HT-29 cells with IC_{50} values of less than 1 μM (Bueno Pérez et

al. 2013; Pan et al. 2012; Pan et al. 2013). Compounds **5** and **9** showed particularly potent activity against HT-29 cells with IC₅₀ values of 0.002 and 0.0007 μ M, respectively, and were comparable in potency to the positive control, paclitaxel, which gave an IC₅₀ value of 0.001 μ M (Pan et al. 2013). In addition, compounds **4–10** and **44–45** were selective for HT-29 cells when compared with CCD-112CoN normal human colon cells (Bueno Pérez et al. 2013; Pan et al. 2012). In a collaborative study, cucurbitacin D (**36**) was found to potently inhibit cell growth of neurofibromatosis type 2 (NF2)-deficient mouse schwannoma (Sch10545) and human benign meningioma (Ben-Men-1) cells, with IC₅₀ values of 0.8 and 0.2 μ M, respectively (Spear et al. 2013). It was also found that compound **36** inhibited the expression of cyclins A, B, and E, in addition to reducing the levels of phosphorylated AKT and PRAS40 (Spear et al. 2013). The known quassinoids bruceantin (**17**) and bruceine A (**18**) were highly active against the human cancer cell lines Lu1 (lung), LNCaP (hormone-dependent prostate), and MCF-7 (breast), with IC₅₀ values in the nanomolar range, whereas rotenoids **44–45** exhibited significant growth inhibition against 697 human acute lymphoblastic leukemia cells with IC₅₀ values of less than 1 μ M (Bueno Pérez et al. 2013; Pan et al. 2009).

Prolonged activation of nuclear factor κ B (NF- κ B) and proteasome activity, as well as decreased apoptosis, are some of the molecular changes directly correlated with oncogenesis (Deng et al. 2009; Gatti et al. 2013; Kim et al. 2006). Therefore, the inhibition of NF- κ B activation, proteasome inhibition and loss of mitochondrial transmembrane potential (MTP), associated with cell death, were also investigated. The activation of NF- κ B (p65) was strongly inhibited by compound **4** (IC₅₀ value of 0.3 μ M) as well as **7** and **12** (both with IC₅₀ values of 0.005 μ M), in addition to elaeocarpin C (**32**) (IC₅₀ value of 2.4 μ M) (Pan et al. 2012; Pan et al. 2013). The coumarin, barbatumol A (**16**), and the xanthone, α -mangostin (**21**), potently induced apoptosis through the disruption of mitochondrial transmembrane potential, with IC₅₀ values of 0.5 and 0.2 μ M, respectively (Pan et al. 2011; Ren et al. 2011).

The cytotoxicities of bruceantin (**17**) and bruceine A (**18**) against MCF-7 cells were enhanced about 10-fold when combined with the flavonolignan (–)-hydnocarpin (**19**), isolated from *Brucea javanica* (Pan et al. 2009). In a similar way, (–)-hydnocarpin (**19**) at 10 μ M also produced a significant potentiating effect in vincristine cytotoxicity at concentrations of 1.5 and 0.75 nM against 697 cells resulting in 83% ($p = 0.03$) and 61% ($p < 0.0001$) increase in growth inhibition, respectively, when compared to vincristine treatment alone. Furthermore, (–)-hydnocarpin (**19**), at doses of 10 and 5 μ M, was able to reverse resistance to vincristine treatment, causing 72% ($p = 0.0001$) and 41% ($p = 0.03$) growth inhibition, respectively, in 697-R human acute lymphoblastic leukemia multidrug-resistant cells overexpressing P-glycoprotein (P-gp) (Bueno Pérez et al. 2012).

In addition to the new flavanones **40–42** with potential cancer chemopreventive activity, a known flavanone (**43**), a rotenoid, tephrosin (**46**), and a chalcone, (+)-tephropurpurin (**47**), isolated from *I. spicata*, together with a semi-synthetic chalcone, (+)-tephrosone (**48**), were active with CD values of less than 10 μ M and low toxicity to Hepa 1c1c7 cells with IC₅₀ values greater than 10 μ M (Bueno Pérez et al. 2013). Moreover, tephrosin (**46**) showed slightly more potent induction activity than the positive control, L-sulforaphane with a CD

value of 0.4 μM , and lower toxicity to Hepa 1c1c7 cells, with an IC_{50} value of 13.9 μM and a CI value of 35.3 (Bueno Pérez et al. 2013).

The mechanism by which dichamanetin (**57**), a *C*-benzylated flavanone isolated from *Piper sarmentosum*, exerts its cytotoxic action was investigated using HT-29, MDA-MB-231 human breast cancer and PC3 human prostate cancer cell lines (Matthew et al. 2011). Dichamanetin (**57**) at 25 μM produced cell cycle arrest, particularly in MDA-MB-231 cells, causing inhibition of cell cycle progression past G1 in about 70% of treated cells (Matthew et al. 2011). Activation of NF- κB has been linked to I κB kinase enzyme (IKK) complex, which regulates the NF- κB signal cascade (Kim et al. 2006). Compound **57** inhibited both IKK and NF- κB expression (Matthew et al. 2011). In addition, dichamanetin (**57**) binds to DNA and affects the mitochondrial transmembrane potential (Matthew et al. 2011).

The ultimate goal of our project is the discovery of new antitumor agents from tropical plants, so therefore promising cytotoxic lead compounds with high selectivity towards cancer cells are tested further in a rapid, efficient and cost effective in vivo system, the hollow fiber assay (Mi et al. 2009; Pearce et al. 2012). The in vivo hollow fiber assays were performed using three human cancer cell lines (HT-29, MCF-7 and MDA-MB-435 melanoma) enclosed in hollow fibers and implanted intraperitoneally (ip) and subcutaneously (sc) in immunodeficient mice (Mi et al. 2009). The new compound, elaeocarpucin C (**32**), and the known compounds, 3,6-di-*O*-acetyl- α -mangostin (**26**) as well as *cis*-(6 α ,12 α)-hydroxyrotenone (**44**), were chosen for in vivo testing due to their high cytotoxic potencies and high selectivities against HT-29 cells (Bueno Pérez et al. 2013; Pan et al. 2012; Ren et al. 2011). Semi-synthesis of elaeocarpucin C (**32**) was performed using a closely related 16,23-epoxycucurbitacin analogue to produce sufficient amounts of this potent compound (IC_{50} value of 0.4 μM against HT-29 cells) for evaluation in the hollow fiber assay (Pan et al. 2012). The known compounds, 3,6-di-*O*-acetyl- α -mangostin (**26**) and *cis*-(6 α ,12 α)-hydroxyrotenone (**44**) also exhibited potent in vitro cytotoxicities, with IC_{50} values of 1.0 and 0.1 μM , respectively, against HT-29 cells (Bueno Pérez et al. 2013; Ren et al. 2011). Compounds **26** and **32** produced no cell growth inhibition in the hollow fiber assay with any of the cell lines or doses under study (Pan et al. 2012; Ren et al. 2011). *cis*-(6 α ,12 α)-Hydroxyrotenone (**44**) was lethal to mice at the high doses of 30, 15, 10 and 5 mg/kg. Therefore, compound **44** was further assayed at the low doses of 2, 1, and 0.5 mg/kg, but this resulted in only a 20% reduction in cell growth for MDA-MB-435 cells at a dose of 2 mg/kg (Bueno Pérez et al. 2013).

Conclusions

The results of this collaborative research project show that tropical plants continue to provide numerous chemically diverse secondary metabolites with activity and selectivity for cancer cells. Some of these natural product lead candidates discovered through bioactivity-guided fractionation might serve as scaffolds for synthetic modifications to potentially develop them as new anticancer agents. The work in this project has been predicated on the collaborative efforts of a multidisciplinary team comprised of biologists, natural products chemists, and plant taxonomists.

Acknowledgments

This research was funded by grant P01 CA125066 (awarded to A. D. K.) from the National Cancer Institute, NIH, Bethesda, MD. Additional support was awarded to C. B. N. and L. B. P. from NIH grant T32 GM008512, as part of the Chemistry-Biology Interface Program (CBIP), and to P. C. S. and L. B. P. from the Raymond W. Doskotch Fellowship in Natural Products Chemistry from the College of Pharmacy, The Ohio State University, as well as a pre-doctoral fellowship awarded to C. B. N. from the American Foundation for Pharmaceutical Education (AFPE). All plants were collected under the terms of agreement between the University of Illinois at Chicago and the Institute of Ecology and Biological Resources of the Vietnam Academy of Science and Technology, Hanoi, Vietnam. Particular thanks are due to the Directors of Hoanglien National Park, Hon Ba Forest Reserve, Kego Nature Reserve Park, and Nui Chua National Park for granting permission to collect the plants specimens, and to the Director of IEBR for overseeing the field operation in the plant collections.

References

- Abd El-Razek MH, Mohamed AE-HH, Ahmed AA. Prenylated flavonoids from *Tephrosia apollinea*. *Heterocycles*. 2007; 71:2477–2490.
- American Cancer Society. Cancer facts & figures 2013. Atlanta: American Cancer Society; 2013. <http://www.cancer.org/acs/groups/content/@epidemiologysurveillance/documents/document/acspc-036845.pdf> [Accessed: Sept 2013]
- Appendino, G.; Fontana, G.; Pollastro, F. Natural products drug discovery. In: Verpoorte, R., editor. *Comprehensive natural products chemistry II*. Vol. 3. Elsevier; Oxford, UK: 2010. p. 205-236.
- Bailly C. Ready for a comeback of natural products in oncology. *Biochem Pharmacol*. 2009; 77:1447–1457. [PubMed: 19161987]
- Bueno Pérez L, Pan L, Sass EJ, Gupta SV, Lehman A, Kinghorn AD, Lucas DM. Potentiating effect of the flavonolignan (–)-hydnocarpin in combination with vincristine in a sensitive and P-gp-expressing acute lymphoblastic leukemia cell line. *Phytother Res*. 2012;10.1002/ptr.4903
- Bueno Pérez L, Li J, Lantvit DD, Pan L, Ninh TN, Chai H-B, Soejarto DD, Swanson SM, Lucas DM, Kinghorn AD. Bioactive constituents of *Indigofera spicata*. *J Nat Prod*. 2013; 76:1498–1504. [PubMed: 23895019]
- Chen ST, Dou J, Temple R, Agarwal R, Wu K-M, Walker S. New therapies from old medicines. *Nat Biotechnol*. 2008; 26:1077–1083. [PubMed: 18846070]
- Cragg GM, Grothaus PG, Newman DJ. Impact of natural products on developing new anti-cancer agents. *Chem Rev*. 2009; 109:3012–3043. [PubMed: 19422222]
- Deng Y, Balunas MJ, Kim J-A, Lantvit DD, Chin Y-W, Chai H-B, Sugiarso S, Kardono LBS, Fong HHS, Pezzuto JM, Swanson SM, Carcache de Blanco EJ, Kinghorn AD. Bioactive 5,6-dihydro- α -pyrone derivatives from *Hyptis brevipes*. *J Nat Prod*. 2009; 72:1165–1169. [PubMed: 19422206]
- Dou, J. Botanical new drugs: from INDs to NDAs. *Planta Med*; Presentation at the 54th Annual Meeting of the American Society of Pharmacognosy; St Louis, MO. 2013. p. 836
- El-Elimat T, Zhang X, Jarjoura D, Moy FJ, Orjala J, Kinghorn AD, Pearce CJ, Oberlies NH. Chemical diversity of metabolites from fungi, cyanobacteria, and plants relative to FDA-approved anticancer agents. *ACS Med Chem Lett*. 2012; 3:645–649. [PubMed: 22993669]
- Feher M, Schmidt JM. Property distributions: differences between drugs, natural products, and molecules from combinatorial chemistry. *J Chem Inf Comput Sci*. 2003; 43:218–227. [PubMed: 12546556]
- Gatti L, Zuco V, Zaffaroni N, Perego P. Drug combinations with proteasome inhibitors in antitumor therapy. *Curr Pharm Des*. 2013; 19:4094–4114. [PubMed: 23181571]
- Gordaliza M. Natural products as leads to anticancer drugs. *Clin Transl Oncol*. 2007; 9:767–776. [PubMed: 18158980]
- Inada A, Sorano T, Murata H, Inatomi Y, Darnaedy D, Nakanishi T. Diamide derivatives and cycloartanes from the leaves of *Aglaia elliptica*. *Chem Pharm Bull*. 2001; 49:1226–1228. [PubMed: 11558621]
- Kim HJ, Hawke N, Baldwin AS. NF- κ B and IKK as therapeutic targets in cancer. *Cell Death Differ*. 2006; 13:738–747. [PubMed: 16485028]

- Kinghorn AD, Carcache de Blanco EJ, Chai H-B, Orjala J, Farnsworth NR, Soejarto DD, Oberlies NH, Wani MC, Kroll DJ, Pearce CJ, Swanson SM, Kramer RA, Rose WC, Fairchild CR, Vite GD, Emanuel S, Jarjoura D, Cope FO. Discovery of anticancer agents of diverse natural origin. *Pure Appl Chem*. 2009; 81:1051–1063. [PubMed: 20046887]
- Kinghorn AD, Pan L, Fletcher JN, Chai H-B. The relevance of higher plants in lead compound discovery programs. *J Nat Prod*. 2011; 74:1539–1555. [PubMed: 21650152]
- Kingston DGI. Modern natural products drug discovery and its relevance to biodiversity conservation. *J Nat Prod*. 2011; 74:496–511. [PubMed: 21138324]
- Kingston DGI, Tamarkin L, Paciotti GF. Conformationally constrained and nanoparticle-targeted paclitaxels. *Pure Appl Chem*. 2012; 84:1455–1467.
- Lachance H, Wetzel S, Kumar K, Waldmann H. Charting, navigating, and populating natural product chemical space for drug discovery. *J Med Chem*. 2012; 55:5989–6001. [PubMed: 22537178]
- Loub WD, Farnsworth NR, Soejarto DD, Quinn ML. NAPRALERT: Computer handling of natural product research data. *J Chem Inform Comp Sci*. 1985; 25:99–103.
- Lucas DM, Still PC, Bueno Perez L, Grever MR, Kinghorn AD. Potential of plant-derived natural products in the treatment of leukemia and lymphoma. *Curr Drug Targets*. 2010; 11:812–822. [PubMed: 20370646]
- Malcolm JR, Liu C, Neilson RP, Hansen L, Hannah L. Global warming and extinctions of endemic species from biodiversity hotspots. *Conserv Biol*. 2006; 20:538–548. [PubMed: 16903114]
- Matthew, S.; Pan, L.; Shen, Q.; Kinghorn, AD.; Swanson, SM.; Carcache de Blanco, EJ. Dichamanetin, a C-benzylated flavonoid from *Piper sarmentosum* inhibits cell growth and induces G1 cell cycle arrest in cancer cells through mitochondrial-mediated apoptosis. Presentation at the 102nd Annual Meeting of the American Association of Cancer Research; Orlando, FL. April 2–April 6; 2011.
- Mi Q, Pezzuto JM, Farnsworth NR, Wani MC, Kinghorn AD, Swanson SM. Use of the in vivo hollow fiber assay in natural products drug discovery. *J Nat Prod*. 2009; 72:573–580. [PubMed: 19161316]
- Newman DJ, Cragg GM. Natural products as sources of new drugs over the 30 years from 1981 to 2010. *J Nat Prod*. 2012; 75:311–335. [PubMed: 22316239]
- Newman DJ, Giddings L-A. Natural products as leads to antitumor drugs. *Phytochem Rev*. 2013; 10:1007/s1111-013-9292-6
- Orjala, J.; Oberlies, NH.; Pearce, CJ.; Swanson, SM.; Kinghorn, AD. Discovery of potential anticancer agents from aquatic cyanobacteria, filamentous fungi, and tropical plants. In: Tringali, C., editor. *Bioactive compounds from natural sources*. 2. Taylor & Francis; London: 2012. p. 37-63. Natural products as lead compounds in drug discovery
- Pan, L.; Matthew, S.; Chai, H-B.; Ninh, TN.; Kleinholz, NM.; Green-Church, KB.; Soejarto, DD.; Carcache de Blanco, EJ.; Kinghorn, AD. Isolation and analysis of aristolochic acid I and other compounds from the roots and other plant parts of *Antidesma bunius*. Presentation at the 52nd Annual Meeting of the American Society of Pharmacognosy; San Diego, CA. July 30–August 3; 2011.
- Pan L, Chin Y-W, Chai H-B, Ninh TN, Soejarto DD, Kinghorn AD. Activity-guided isolation of cytotoxic constituents of *Brucea javanica* collected in Vietnam. *Bioorg Med Chem*. 2009; 17:2219–2224. *ibid*. 19:1562 (2011). [PubMed: 19026551]
- Pan L, Matthew S, Lantvit DD, Zhang X, Ninh TN, Chai H-B, Carcache de Blanco EJ, Soejarto DD, Swanson SM, Kinghorn AD. Bioassay-guided isolation of constituents of *Piper sarmentosum* using a mitochondrial transmembrane potential assay. *J Nat Prod*. 2011; 74:2193–2199. [PubMed: 21973101]
- Pan L, Chai H-B, Kinghorn AD. Discovery of new anticancer agents from higher plants. *Front Biosci (Schol Ed)*. 2012; 4:142–156. [PubMed: 22202049]
- Pan L, Yong Y, Muñoz Acuña U, Deng Y, Lantvit DD, Ninh TN, Chai H-B, Carcache de Blanco EJ, Soejarto DD, Swanson SM, Kinghorn AD. Isolation, structure elucidation, and biological evaluation of 16,23-epoxycucurbitacin constituents of *Elaeocarpus chinensis*. *J Nat Prod*. 2012; 75:444–452. [PubMed: 22239601]

- Pan L, Muñoz Acuña U, Li J, Jena N, Ninh TN, Pannell CM, Chai H-B, Fuchs JR, Carcache de Blanco EJ, Soejarto DD, Kinghorn AD. Bioactive flavaglines and other constituents isolated from *Aglaia perviridis*. J Nat Prod. 2013; 76:394–404. [PubMed: 23301897]
- Pearce, CJ.; Lantvit, DD.; Shen, Q.; Jarjoura, D.; Zhang, X.; Oberlies, NH.; Kroll, DJ.; Wani, MC.; Orjala, J.; Soejarto, DD.; Farnsworth, NR.; Carcache de Blanco, EJ.; Fuchs, JR.; Kinghorn, AD.; Swanson, SM. Use of the hollow fiber assay for discovery of novel anticancer agents from fungi. In: Keller, N.; Turner, G., editors. Fungal secondary metabolism: methods and protocols. Vol. 944. Humana Press/Springer; Totowa, NJ: 2012. p. 267–277.
- Rahier, NJ.; Thomas, CJ.; Hecht, SM. Camptothecin and its analogs. In: Cragg, GM.; Kingston, DGI.; Newman, DJ., editors. Anticancer agents from natural products. CRC Taylor & Francis; Boca Raton: 2005. p. 5–21.
- Rai PK, Lalramnghinglova H. Threatened and less known ethnomedicinal plants of an Indo-Burma hotspot region: conservation implications. Environ Monit Assess. 2011; 178:53–62. [PubMed: 20809385]
- Rao EV, Raju NR. Two flavonoids from *Tephrosia purpurea*. Phytochemistry. 1984; 23:2339–2342.
- Ren Y, Lantvit DD, Carcache de Blanco EJ, Kardono LBS, Riswan S, Chai H-B, Cottrell CE, Farnsworth NR, Swanson SM, Ding Y, Li XC, Marais JPJ, Ferreira D, Kinghorn AD. Proteasome-inhibitory and cytotoxic constituents of *Garcinia lateriflora*: absolute configuration of caged xanthenes. Tetrahedron. 2010; 66:5311–5320. [PubMed: 20730041]
- Ren Y, Matthew S, Lantvit DD, Ninh TN, Chai H-B, Fuchs JR, Soejarto DD, Carcache de Blanco EJ, Swanson SM, Kinghorn AD. Bioactive constituents of the stems of *Cratogeomys cochinchinense* and some semi-synthetic analogues. J Nat Prod. 2011; 74:1117–1125. [PubMed: 21428375]
- Siegel R, Naishadham D. Cancer statistics, 2013. CA Cancer J Clin. 2013; 63:11–30. [PubMed: 23335087]
- Spear SA, Burns SS, Oblinger JL, Ren Y, Pan L, Kinghorn AD, Welling DB, Chang LS. Natural compounds as potential treatments of NF2-deficient schwannoma and meningioma: cucurbitacin D and goyazensolide. Otol Neurotol. 2013; 34:1519–1527. [PubMed: 23928514]
- Still PC, Yi B, González Muskus T, Pan L, Pavlovicz R, Chai H-B, Ninh TN, Li C, Soejarto DD, McKay D, Kinghorn AD. Alkaloids from *Microcos paniculata* with cytotoxic and nicotinic receptor antagonistic activities. J Nat Prod. 2013; 76:243–249. [PubMed: 23327794]
- Thoison O, Fahy J, Dumontet V, Chiaroni A, Riche C, Tri MV, Sévenet T. Cytotoxic prenylxanthenes from *Garcinia bracteata*. J Nat Prod. 2000; 63:441–446. [PubMed: 10785410]
- Waterman PG, Khalid SA. The major flavonoids of the seed of *Tephrosia apollinea*. Phytochemistry. 1980; 19:909–915.
- Zhang Y, Talalay P, Cho C-G, Posner GH. A major inducer of anticarcinogenic protective enzymes from broccoli: isolation and elucidation of structure. Proc Natl Acad Sci USA. 1992; 89:2399–2403. [PubMed: 1549603]

Fig. 1.

Comparison of the types (origin) of all new U. S. FDA approved drugs from the calendar year 2012 ($n = 40$). The Center for Drug Evaluation and Research of the FDA reported only 39 new molecular entity approvals in its report for that calendar year (<http://www.fda.gov/Drugs/DevelopmentApprovalProcess/DrugInnovation/ucm336115.htm>), seemingly omitting the July, 2012 approval of the marine natural product derived drug Vascepa® (eicosapentaenoic acid methyl ester), which is included above. For simplicity, bacterial and human proteins approved as biological drugs were considered as bacterial and animal natural products, respectively.

- 2*** R₁ = OH R₂ = H R₃ = OCH₃
3* R₁ = OH R₂ = COOCH₃ R₃ = OCH₃
4 R₁ = OH R₂ = H R₃ = OH
5 R₁ = OH R₂ = COOCH₃ R₃ = OH
6 R₁ = OCHO R₂ = COOCH₃ R₃ = OH

- 7** R₁ = OH R₂ = H R₃ = OH
8 R₁ = OH R₂ = COOCH₃ R₃ = OH
9 R₁ = OH R₂ = CONH₂ R₃ = OH
10 R₁ = OCHO R₂ = COOCH₃ R₃ = OH
11 R₁ = OH R₂ = COOCH₃ R₃ = OCH₃

- 13** R₁ = H, R₂ = H
14 R₁ = CH₃, R₂ = H
15 R₁ = CH₃, R₂ = OCH₃

- 17** R = (*E*)-3,4-Dimethyl-2-pentenyl
18 R = Isopentanyl

- 21** R = OCH₃
22 R = OH

- 23** R = H
24* R = CH₃

25 $R_1 = \text{OH}$, $R_2 = \text{OH}$

26 $R_1 = \text{OAc}$, $R_2 = \text{OH}$

27 $R_1 = \text{OAc}$, $R_2 = \text{OAc}$

28

29

30 $R = \text{OCOH}$

31 $R = \text{OH}$

32* $R_1 = R_2 = R_3 = \text{H}$ $R_4 =$

33* $R_1 = R_2 = R_3 = \text{H}$ $R_4 =$

34* $R_1 = R_2 = \text{H}$, $R_3 = \text{OH}$ $R_4 =$

35* $R_1, R_2 = \text{O}$, $R_3 = \text{H}$ $R_4 =$

36 $R_1 = \text{H}$, $R_2 = \text{OH}$, $R_3, R_4 = \text{O}$, $R_5 = \text{OH}$

37 $R_1, R_2 = \text{O}$, $R_3 = \text{H}$, $R_4 = \text{OH}$, $R_5 = \text{OH}$

38 $R_1 = \text{H}$, $R_2 = \text{OH}$, $R_3 = \text{OH}$, $R_4 = \text{H}$, $R_5 = \text{OAc}$

39

40*

41*

42*

43

44 $R = \text{OH}$

45 $R = \text{H}$

46

47 $R_1 = \text{OCOCH}_3$ $R_2 = \text{OCH}_3$

48 $R_1 = \text{OH}$ $R_2 = \text{H}$

49*

Fig. 2.
Structures of the plant-derived lead compounds obtained from 2009 to the present. *New compound.

Table 1Plant natural products and derivatives approved by the U.S. FDA from 2011 to mid-2013^a

Year Approved	Generic Name	Natural or Lead Compound	Trade Name	Indication
2011	ioflupane I-123 ^b	cocaine	Datscan®	brain imaging in suspected Parkinsonian syndromes
2011	gabapentin enacarbil ^b	gamma-aminobutyric acid (GABA)	Horizant®	restless leg syndrome
2012	ingenol mebutate ^b	ingenol-3-angelate	Picato®	actinic keratosis
2012	lorcaserin hydrochloride ^b	ephedrine	Belviq®	obesity
2012	omacetaxine mepesuccinate ^b	homoharringtonine	Synribo®	chronic myeloid leukemia
2012	crofelemer ^c	<i>Croton lechleri</i> oligomeric proanthocyanidins	Fulyzaq®	HIV/AIDS anti-retroviral-associated diarrhea
2013	ado-trastuzumab emtansine (protein-bound) ^b	maytansine	Kadcyla®	breast cancer
2013	ospemifene ^b	phytoestrogens	Osphena®	menopause-associated dyspareunia

^a Information taken from the Drugs@FDA database (<http://www.accessdata.fda.gov/scripts/cder/drugsatfda>).^b New molecular entity.^c Approved as a mixture of compounds as a “botanical drug”.

Table 2Summary of cytotoxic and mechanism-based activities for plant-derived compounds^a

Compound	Plant Species (Family)	Cytotoxicity Assay Cell line, IC ₅₀ value (μM)	Mechanism-based Assay Cell line, Assay, IC ₅₀ value (μM)	Ref.
1^b	<i>Aglaia perviridis</i> Hiern (Meliaceae)	HT-29, 0.5	HeLa, NF-KB (p65), 2.4	Pan et al. 2013
2^b		HT-29, 1.0	*	
3^b		HT-29, 4.7	*	
4		HT-29, 0.007	HeLa, NF-KB (p65), 0.3	
5		HT-29, 0.002	HeLa, NF-KB (p65), 3.5	
6		HT-29, 0.06	*	
7		HT-29, 0.0007	HeLa, NF-KB (p65), 0.005	
8		HT-29, 0.01	*	
9		HT-29, 0.02	*	
10		HT-29, 0.02	*	
11		HT-29, 4.9	*	
12		*	HeLa, NF-KB (p65), 0.005	
13	<i>Antidesma buniis</i> (L.) Spreng (Euphorbiaceae)	HT-29, 7.6	*	Pan et al. 2011
14		*	HT-29, MTP, 1.5	
15		*	HT-29, MTP, 1.8	
16		*	HT-29, MTP, 0.5	
17	<i>Brucea javanica</i> (L.) Merr. (Simaroubaceae)	Lu1, 0.01; LNCaP, 0.01; MCF-7, 0.002	*	Pan et al. 2009
18		Lu1, 0.1; LNCaP, 0.1; MCF-7, 0.02	*	
20^b	<i>Cratoxylum cochinchinense</i> (Lour.) Bl. (Clusiaceae)	HT-29, 5.8	HT-29, MTP, 3.3	Ren et al. 2011
21		HT-29, 4.1	HT-29, MTP, 0.2	
22		HT-29, 4.0	*	
23		*	HT-29, MTP, 1.4	
24^b		*	HT-29, MTP, 0.9	
25		HT-29, 8.8	*	
26		HT-29, 1.0	HT-29, MTP, 1.8	
27		HT-29, 6.0	*	
28		*	HeLa, NF-KB (p65), 2.9	
29		HT-29, 1.9	*	
30		HT-29, 4.4	*	
31		HT-29, 4.4	*	
32^b	<i>Elaeocarpus chinensis</i> (Gardner & Champ.) Hook.f. ex Benth. (Elaeocarpaceae)	HT-29, 0.4	*	Pan et al. 2012 Spear et al. 2013
33^b		HT-29, 5.6	*	
34^b		HT-29, 2.9	*	
35^b		HT-29, 2.7	*	

Compound	Plant Species (Family)	Cytotoxicity Assay Cell line, IC ₅₀ value (μM)	Mechanism-based Assay Cell line, Assay, IC ₅₀ value (μM)	Ref.
36		HT-29, 0.1	*	
		Sch10545, 0.8	*	
		Ben-Men-1, 0.2	*	
37		HT-29, 0.04	*	
38		HT-29, 0.5	*	
39		HT-29, 0.2	*	
44	<i>Indigofera spicata</i> Forssk. (Fabaceae)	HT-29, 0.1; 697 ^{**} , 0.7 and 0.5; Raji ^{**} , 5.6 and 2.4	*	Bueno Pérez et al. 2013
45		HT-29, 0.3; 697 ^{**} , 0.3; Raji ^{**} , 4.2 and 1.1	*	
46		697 ^{**} , 5.4 and 9.0	*	
49 ^b	<i>Microcos paniculata</i> L. (Malvaceae)	HT-29, 6.8	*	Still et al. 2013
50 ^b	<i>Piper sarmentosum</i> Roxb. (Piperaceae)	*	HT-29, MTP, 3.7	Pan et al. 2011
51 ^b		*	HT-29, MTP, 6.8	
52 ^b		*	HT-29, MTP, 2.5	
53 ^b		*	HT-29, MTP, 3.9	
54		*	HL-60, proteasome, 2.9	
55		*	HT-29, MTP, 4.6	
56		*	HT-29, MTP, 7.7	
57		*	HT-29, MTP, 3.6	
58		*	HT-29, MTP, 1.6; HL-60, proteasome, 3.5	
59		*	HT-29, MTP, 2.3	

^aKey to cell lines used: Ben-Men-1, human benign meningioma; HeLa, human cervix adenocarcinoma; HL-60, human leukemia; HT-29, human colon cancer; LNCaP, hormone-dependent human prostate cancer; Lu1, human lung cancer; MCF-7, human breast cancer; Raji, human Burkitt's lymphoma; Sch10545, NF2-deficient mouse schwannoma; 697, human acute lymphoblastic leukemia.

^bNew compound.

* Inactive or not tested.

** Results read at 48 and 72 h incubation, respectively.