

## CHAPTER 22

### THE SCHWEINFURTHINS

*Issues in development of a plant-derived anticancer lead*

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**Abstract.** The development of a natural product into a pharmaceutical drug product can follow a long and tortuous path in the best of cases. This chapter highlights issues that have arisen in the development of the schweinfurthins, a compound class discovered from an African plant, *Macaranga schweinfurthii*, as a potential cancer drug lead. Resupply of the plant from Cameroon, synthetic access to this series of compounds, and investigation of its mechanism of action, have all played major roles in the story to date. An interdisciplinary network of scientific collaboration has been assembled to make advancement possible.

**Keywords:** schweinfurthin; cancer; *Macaranga*; *Euphorbiaceae*; stilbene; Cameroon; drug development

#### INTRODUCTION

For the past fifty years the United States National Cancer Institute (NCI) has provided a resource for the pre-clinical screening of compounds and materials submitted by grantees, contractors, pharmaceutical and chemical companies and other scientists and institutions, public and private, worldwide, and has played a major role in the discovery and development of many of the available commercial and investigational anticancer agents. Initially, most of the materials screened were pure compounds of synthetic origin, but the program also recognized that natural products were an excellent source of complex chemical structures with a wide variety of biological activities. During this period, more than 500,000 chemicals, both synthetic and natural, were screened for anti-tumour activity. In addition, from

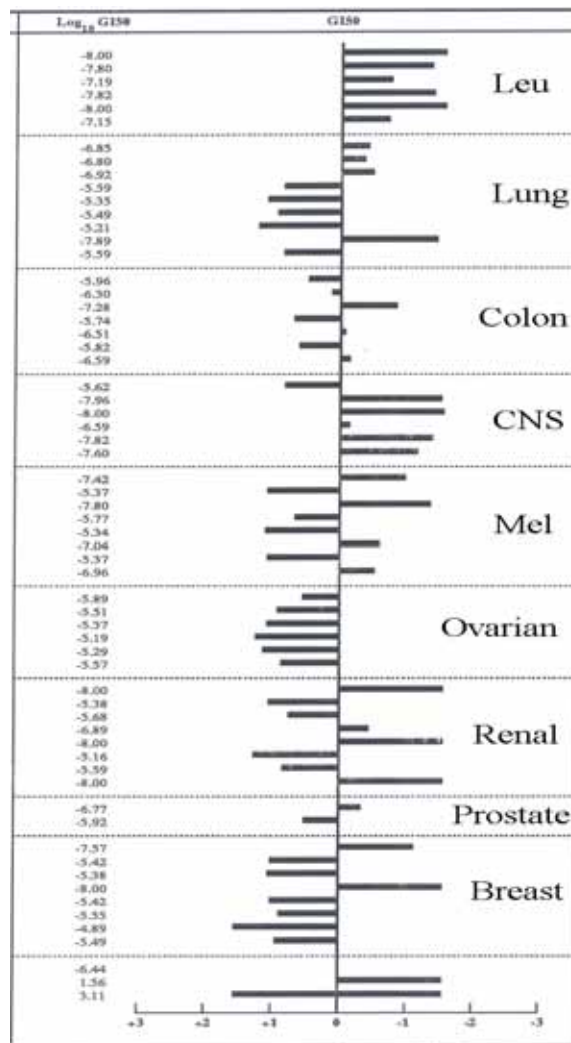
1960 to 1982 over 180,000 microbial-derived, some 16,000 marine-organism-derived and over 114,000 plant-derived extracts were screened for anti-tumour activity in intact animal models. Major plant-derived discoveries from this period include taxol<sup>®</sup> from the Pacific yew (*Taxus brevifolia*) and camptothecin from the Chinese tree, *Camptotheca acuminata*. Both of these compounds were isolated by the team of Wall and Wani at Research Triangle Institute [reviewed by Cragg and Newman (2004)], and have been developed into clinically effective drugs such as paclitaxel, docetaxel, topotecan and irinotecan.

In 1986, new contracts for the cultivation and extraction of fungi and cyanobacteria and for the collection of marine invertebrates and terrestrial plants were initiated. Terrestrial-plant collections have been carried out in over 25 countries in tropical and subtropical regions worldwide through contracts with the Missouri Botanical Garden (Africa and Madagascar), the New York Botanical Garden (Central and South America) and the University of Illinois at Chicago (Southeast Asia), and were later expanded to United States territories through contracts with the Morton Arboretum and World Botanical Associates. In addition to Madagascar, the African collections have been performed in Cameroon, Central African Republic, Gabon, Ghana and Tanzania. In total, over 65,000 plant samples representing over 16,000 species have been collected, and the organic and aqueous extracts prepared from these samples are stored at -20°C in the NCI Natural Products Repository in Frederick, Maryland. These extracts have been utilized in the *in vitro* 60-cell cancer screen that began testing in 1989, and which replaced the whole animal primary screening of the previous decades.

In carrying out the contract plant collections, the NCI contractors mentioned above have worked closely with qualified organizations and scientists in each of the source countries. Botanists from source-country organizations have collaborated in field collection activities and taxonomic identification, and their knowledge of local species and conditions has been indispensable to the success of the NCI collection operations. These collaborations have provided support for expanded research activities by source-country biologists, and the deposition of voucher specimens of each species collected in the national herbarium or repository has expanded source-country holdings of their biota. NCI contractors have also provided training opportunities for local personnel through conducting workshops and presentation of lectures.

In addition to work within the source countries, through its Letter of Collection (LOC; <http://ttb.nci.nih.gov/nploc.html>) and agreements based upon it, the NCI has invited scientists nominated by source-country organizations to visit its facilities or equivalent facilities in other approved U.S. organizations for 1-12 months to participate in collaborative natural-products research. Representatives of many of the source countries have also visited the NCI and contractor facilities for shorter periods to discuss collaboration. Another aspect of the LOC dictates terms of benefit-sharing and use of source-country resources in the event that a promising drug candidate is licensed and developed. It should be noted that the formulation of the NCI policies for collaboration and compensation embodied in the Letter of Collection predated the drafting of the United Nations Convention on Biological Diversity in Rio de Janeiro by some four years.

In September 2004, the contract plant collection program was discontinued, but collaborations continue with qualified source-country organizations in the study of their plant resources. In establishing these collaborations, the NCI undertakes to abide by the same policies of collaboration and compensation as specified in the LOC. Memoranda of Understanding (MOUs; <http://ttb.nci.nih.gov/npmou.html>) have been signed with organizations in several source countries.



**Figure 1.** GI<sub>50</sub> mean graph for schweinfurthin A in NCI 60 cancer cell assay. Mid-line of graph represents the mean GI<sub>50</sub> of 0.36 μM (10<sup>-6.44</sup>M). The most sensitive cell line, SF-295, has a GI<sub>50</sub> value of <10 nM, while the least sensitive cell line, BT-549, has a GI<sub>50</sub> value of 13 μM.

## INITIAL BIOLOGICAL ACTIVITY OF SCHWEINFURTHINS

Our interest in *Macaranga* originated in the observation that an organic extract of *Macaranga schweinfurthii* Muell.Arg. (*Euphorbiaceae*) leaves possessed potent (mean 50 % growth inhibition of 2.2  $\mu\text{g/mL}$ ) and differential (>2000-fold) activity in the NCI 60 human tumour-cell assay (Figure 1). The most sensitive cell lines included several brain tumour cell lines, especially the glioblastoma lines SF-295 and SF-539, and a number of lines in other organ panels. The isolation and structure elucidation of the novel cytotoxic constituents, which we named schweinfurthins A and B, was reported previously (Beutler et al. 1998). This chapter reviews progress since that time in the development of the schweinfurthins as anticancer lead compounds.

## SUPPLY ISSUES: RECOLLECTION OF THE PLANT

The original collection of *M. schweinfurthii* leaves was made in the vicinity of Mundemba, Ndian Division, Korup National Park, in South-western Cameroon on March 25, 1987 by Duncan W. Thomas, who was working as a collector for the Missouri Botanical Garden. The collected leaves (425 g) yielded 22 g of organic extract by the conventional percolation method used by the NCI (dichloromethane – methanol 1:1 v/v followed by a methanol rinse). In the 60-cell human tumour-cell assay, this extract showed a mean  $\text{GI}_{50}$  of 2.2  $\mu\text{g/mL}$ . From the total mass we isolated 50 mg of pure schweinfurthin A, as well as 38 mg of the somewhat less active schweinfurthin B and 25 mg of the biologically inactive schweinfurthin C. (Beutler et al. 1998).

From the same plant, 415 g of stem material was collected, yielding only 5 g of organic extract. As the mean  $\text{GI}_{50}$  of this extract in the 60-cell assay was only 21  $\mu\text{g/mL}$ , the sample was not fractionated, since it would be expected to yield only 1-2 mg of schweinfurthin A, assuming that it contained schweinfurthin A as the major active agent.

We arranged a recollection of this species from Cameroon in November of 1998. Due to the difficulty of travelling to the Korup National Park, the recollection of *M. schweinfurthii* was made in the vicinity of Yaounde. Since methylene chloride was expensive and unavailable locally, we settled on hot ethanol extraction; the compounds had demonstrated good solubility in this solvent. However, chemical and cytotoxicity examination of the extract at the NCI detected no schweinfurthins A or B in the samples from this collection. To rule out the possibility that the schweinfurthins were unstable in hot ethanol (though we considered this unlikely) a second collection was made in February of 1999 in Mbalmayo near Yaounde, and extracted at room temperature; however, once more no schweinfurthins A or B were found.

A return to the original site of collection (Korup National Park) in March of 1999, followed by room-temperature extraction with ethanol, finally yielded the desired compounds. However, we were disappointed to find that the yield of schweinfurthins A and B was several-fold lower than in the original collection. In

this recollection we also found one new compound, schweinfurthin D, with similar bioactivity (Beutler et al. 2000).

At this point we began to consider other options for supply. Could these compounds be found in other species? *Macaranga* is a widespread genus in the old world tropics, with a centre of diversity in SE Asia (e.g. Figure 2; see colour pages elsewhere in this book), encompassing approximately 300 species (Webster 1994). NCI collections already in hand included samples from 52 collections of 38 distinct species over the entire geographic range of the genus. However, none of the extracts originating from the other *Macaranga* species demonstrated a cytotoxicity profile consistent with the schweinfurthins. While schweinfurthin-like compounds have not to this date been found in NCI *Macaranga* collections, other reports show that the ability to biosynthesize schweinfurthins is not unique to the single species *M. schweinfurthii*. Indeed, the compound vedelianin was isolated from *M. vedeliana* from the Loyalty Islands, New Caledonia prior to our work, although no bioactivity was attributed to it at the time (Thoison et al. 1992). Mappain, an analog of schweinfurthin C in which neither side chain is cyclized, was isolated from a Philippine species, *M. mappa*, growing in Hawaii (Van der Kaaden et al. 2001). Most recently, the Kingston group has reported isolation of cytotoxic schweinfurthin analogues from a Madagascan species of *Macaranga* (Yoder et al. 2004).

One potential reason for the inconsistent occurrence of schweinfurthins might be that production could be a response to herbivory, or to another type of threat to the plant's well-being. Indeed, the voucher specimen for the initial collection of *M. schweinfurthii* is a large leaf full of holes (Figure 3; see colour pages elsewhere in this book), presumably due to insect damage. Another independent photo of this species on the worldwide web at <http://members.chello.be/sf16063/pauwels/MacaSchw.jpg> shows similarly perforated leaves. It is tempting to speculate that schweinfurthins may serve as a third strategic option for defence of *Macaranga*, in addition to the production of tannins (Lin et al. 1990) or allelochemicals (Tseng et al. 2001), and to defence by ants (myrmecophytism) (Davies et al. 2001; Eck et al. 2001). It will be interesting to determine if these modes of defence are mutually exclusive within the genus, and how they are distributed among its species.

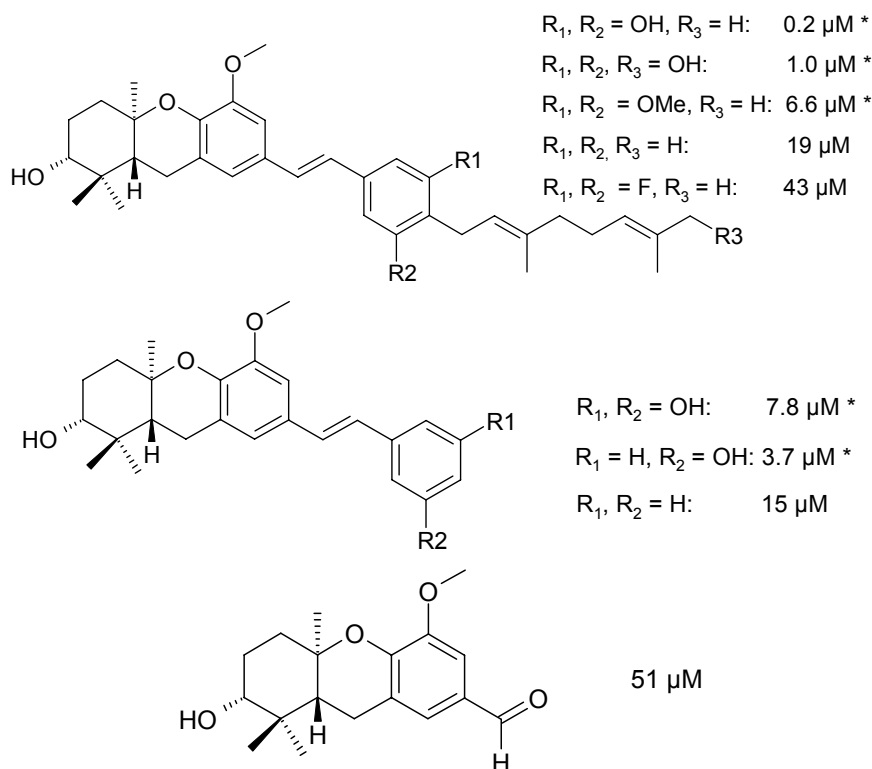
#### SUPPLY ISSUES: SYNTHESIS

With the growing realization that resupply of schweinfurthins from natural sources was not likely to be straightforward, we began a collaboration to explore the total synthesis of schweinfurthins, using chemical technology already developed for synthesis of prenylated aromatic compounds in the Wiemer lab. This initiative promised opportunities to elucidate the undefined absolute stereochemistry of the 'left half' of schweinfurthins A, B and D, and to provide structure-activity information, in addition to supplying more of the natural compounds. Schweinfurthin C, the non-cytotoxic congener, was readily synthesized (Treadwell et al. 1999). In the synthesis of the left half of the tetracyclic schweinfurthins, we encountered difficulty in properly functionalizing the vicinal diol, but fortuitously it was found that 3-oxygenation was not required for cytotoxicity. Indeed, 3-

deoxyschweinfurthin B was twofold more potent than its parent, schweinfurthin B (Neighbors et al. 2005).

We have used this observation as the basis for development of a series of analogues of 3-deoxyschweinfurthin B to explore the structural requirements for the selective cytotoxicity which initially drew our interest (Neighbors et al. in press). Modification of the 'right' half has demonstrated that the prenyl group is not strictly required, though potency is compromised. At least one of the phenolic hydroxyl groups is required for bioactivity. Replacement of both phenols with fluorine completely abolished activity (Figure 4).

In addition, the chiral synthesis of both enantiomers of 3-deoxyschweinfurthin B has led us to hypothesize that natural schweinfurthins possess the *R,R,R*-stereochemistry, since *R,R,R*-3-deoxyschweinfurthin B was approximately threefold more potent than the *S,S,S*-isomer and possessed differential cytotoxicity more highly correlated with the natural compounds. A final word on this stereochemical proposal will require the synthesis of the natural product itself in enantiopure form.



**Figure 4.** Structure of synthetic schweinfurthin analogs and their potency (mean  $GI_{50}$ ) in the NCI 60-cell assay. Compounds marked with an asterisk showed similar patterns of differential cytotoxicity

## MECHANISM-OF-ACTION STUDIES

Compounds have difficulty advancing as drug candidates if the mechanism of action and/or mechanism of toxicity are/is not known. Once these are elucidated, progress is much faster and enthusiasm is much higher. Many examples could be cited but the history of taxol, which was found to bind to polymerized tubulin (Parness and Horwitz 1981), and that of bryostatin 1, found to interact with protein kinase C (Berkow and Kraft 1985; Smith et al. 1985), certainly support this observation.

We used the profile of cytotoxicity of schweinfurthins in the NCI 60-cell screen to explore whether other compounds of known mechanism of action shared their profile (Paull et al. 1995; Monks et al. 1997). No such compounds were identified, but the cephalostatins, large bisteroidal pyrazines from a marine worm (Pettit et al. 1988), stelletins, from marine sponges (Su et al. 1994; McCormick et al. 1996), and the saponin OSW-1 (Mimaki et al. 1997) appeared to possess the same pattern of differential cytotoxicity. While the schweinfurthins are not nearly as potent as the cephalostatins, their simpler structure may facilitate synthetic manipulation to create affinity reagents which can be used to probe their actions on cancer cells. A molecular mechanism of action for the cephalostatins, stelletins and OSW-1 is yet to be determined (but see Dirsch et al. 2003; Komiya et al. 2003; Müller et al. 2005 for preliminary work on cephalostatins), so the suggestion of a common mechanism or pathway does not shed much light on how the schweinfurthins work. Our empirical observations of the action of schweinfurthin on cancer cells indicate that they do not have an obvious effect on the cell cycle (Scudiero unpublished). Similarly, their effect on cell growth is not immediate: a 2-hour exposure has little effect on cell viability, and a full effect requires continuous exposure for 48 hours or more (Beutler et al. 1998). Last, they produce a characteristic spindle-shaped cell morphology at sublethal concentrations (Oku and Fusetani unpublished). Similar results have been obtained in these empirical assays for the cephalostatins, as well as for the stelletins and OSW-1, supporting the idea of a common pathway. An investigation of the molecular mechanism of schweinfurthin action is contemplated once appropriate affinity reagents are available from synthesis.

*IN VIVO* TESTING

The ability to kill or suppress the growth of tumor cells *in vitro* is a necessary property for an anti-tumour drug candidate. However, it must also be able to survive metabolism and excretion for long enough to reach the tumour, to have any possible clinical utility. Therefore, we investigated schweinfurthin A in several *in vivo* mouse cancer models.

As a first step, schweinfurthin A was evaluated in a hollow-fibre *in vivo* efficacy model against the sensitive SF-295 tumour cell line (Hollingshead et al. 1995). For this, tumour cells were packaged into polyvinylidene fluoride (PVDF) hollow fibres (molecular exclusion >500 kDa) and implanted both subcutaneously (sc) and intraperitoneally (ip) into mice. Tumour-cell-bearing mice were treated with schweinfurthin A at doses of 10, 5 or 2.5 mg/kg/dose on one of 3 schedules: daily for 4 days (QDx4), 2 doses given 2 days apart (Q2Dx2), or a single dose (QDx1).

Each schedule of drug treatment was performed by both ip and intravenous (iv) routes. After treatment, the fibres were collected and the anti-proliferative effect of schweinfurthin A was measured by comparison of the viable-cell numbers measured in the treated and control samples. There were statistically significant reductions in viable tumour cell mass in several treatment groups but the greatest activity occurred in the mice receiving schweinfurthin A on the QDx4 treatment schedule by the ip route of administration ( $p < 0.03$ ). This suggests that schweinfurthin A is available in biologically active concentrations following intraperitoneal administration at doses which did not produce detectable toxicity. The finding of greater activity with the most frequent dosing schedule is consistent with the *in vitro* studies that demonstrated greater activity with prolonged exposure to the agent.

The hollow-fibre studies were followed by an efficacy study conducted against sc xenografts of SF-295 cells in athymic mice. Limitations on the availability of test material limited the number of doses given to 4 per mice. In an attempt to spread these over the course of tumour growth, a schedule of Q2Dx4 was selected. At a dose of 9.3 mg/kg there was some reduction in tumour burden in treated mice compared to vehicle-treated controls. Additional studies using more intensive dosing or administration by continuous infusion might well produce greater reductions in tumour burden than was seen in this single xenograft experiment. In any case, the evidence supports a conclusion that schweinfurthin A has the potential to impact tumour growth in the physiologic compartments of mammals.

### CONCLUSIONS

The schweinfurthins are a promising class of anti-tumour drug candidates whose mechanism of action remains unknown but enticing. If the mechanism of action can be defined, it is likely to represent a novel approach to control of cancer cell growth. Further investment in structural optimization is likely to lead to analogues worthy of drug development.

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