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2. Role of plants in Cancer Drug Discovery- A Review

2.1.1 Plants as a source of anticancer agents

Plants have played a dominant role in the development of sophisticated traditional medicine systems. The WHO estimates that approximately 80% of the population in some Asian and African countries depend on traditional medicine for primary health care. Plant products, however, also play an important secondary role in the health care sectors of developed countries, with 70–80% of populations of developed countries having used some form of alternative or complementary medicine (e.g. acupuncture). Herbal treatments are the most popular form of traditional medicine, and are highly lucrative in the international market. The global market for herbal products is expected to reach $5 Trillion by 2050 (Anand and Neetu, 2011).

Plants products have a long history of use in the treatment of cancer. Hartwell in his review of plants used against cancer lists more than 3000 plant species (Hartwell, 1982). Plant based drug discovery has resulted in the development of many anticancer drugs currently in clinical use. Besides this it also provides a platform for design of novel and safe drugs through proper understanding of the complex synergistic interaction of various constituents of anticancer herbs (Larkin, 1983; Saxe, 1987). There are four major structural classifications of plant-derived anticancerous compounds, namely vinca alkaloids, epipodophyllotoxin lignans, taxane diterpenoids and camptothecin quinoline alkaloid derivatives. These substances embrace some of the most exciting new chemotherapeutic agents currently available for use in a clinical setting.

2.1.2 Plant derived anticancer agents in clinical development

*Dysoxylum binectariferum* hook. f., Meliaceae family, rohitukine was isolated and this flavonoid structure formed the basis for a novel synthetic flavonoid structure, flavopiridol. During a structure activity study over 100 synthetic analogs were synthesized. These analogs were tested against a series of breast and lung carcinoma cell lines, in the course of these studies it was found that they have tyrosine kinase activity and potent growth inhibitory activity (Cragg and Newman, 2005). Flavopiridol showed the most potent activity. *In vivo* (in mice) broad spectrum activity was found against human tumour xenografts. The National
Cancer Institute (NCI) then selected it for preclinical and clinical studies in collaboration with the Hoechst Company; currently it is in Phase I and Phase II clinical trials. Flavopiridol is effectively used alone or in combination with other anticancer agents to treat a broad range of tumours, leukemias, lymphomas and solid tumours (Cragg and Newman, 2005).

In the 1970s the NCI and United States Department of Agriculture (USDA) were working together with the South African Botanical Research Institute on a random collection program. Combretastatins, a family of stilbenes, were isolated from the South African Combretum caffrum (Eckl. & Zeyh.) Kuntze, which was collected as part of that random collection program. The genera Combretum and Terminalia both belong to the family Combretaceae that are used for malaria, hepatitis and a variety of other diseases in Indian and African traditional medicine. Reportedly, several of the Terminalia species have been used for cancer treatment. The combretastatins act as anti-angiogenic agents, they cause tumour necrosis through vascular shutdown in tumours (Cragg and Newman, 2005). One of the water-soluble analogs of the combretastatins, A4 phosphate (CA4), has shown promising activity in early clinical trials, and now several mimics are being developed of which three all in clinical trials and another 11 in the preclinical development.

By combining medicinal and combinatorial chemistry a multitude of analogs were synthesized from this chemical class that served as a model which had a relatively simple natural product structure. All of them containing the crucial trimethoxy aryl moiety linked to substituted aromatic moieties through a variety of two or three atom bridges together with heterocyclic rings and sulfonamides (Li and Sham, 2002).

Olomucine was first isolated from the cotyledons of Raphanus sativus L. (Brassicaceae) (radish) (Meijer & Raymond, 2003). Olomucine inhibit cycline-dependent kinases (Ddk), proteins which play a major role in cell cycle progression (Cragg and Newman, 2005).

Roscovitine (derived from olomucine) is a more potent inhibitor that resulted from chemical modification. In Europe, roscovitine is currently in Phase II clinical trials and further development was also taking place within this series of olomucine derived compounds which led to the development of ‘purvalanols’ (Cragg and Newman, 2005; Chang et al. 1999).

Purvalanols is currently undergoing preclinical development because they are even more potent than the natural product olomucine and its synthetic derivative roscovitine.
Today a number of plant derived anticancer agents are available in the market. Their mode of action and target are well known (Table 2.1).

**Table 2.1: Summary of anticancer agents derived from natural products (Fang, 2006)**

<table>
<thead>
<tr>
<th>Compounds</th>
<th>Drug name</th>
<th>Source</th>
<th>Cancer use</th>
<th>Mode of action</th>
<th>Arrested cell cycle</th>
</tr>
</thead>
<tbody>
<tr>
<td>Doxorubicin</td>
<td>Adriamycin® Rubex®</td>
<td><em>Streptomyces peucetius</em> var. <em>caesius</em> (Microbe)</td>
<td>Lymphoma, breast, ovary, lung and sarcomas</td>
<td>Topoisomerase II inhibition and DNA binding</td>
<td>G2/M phase</td>
</tr>
<tr>
<td>Etoposide/Teniposide</td>
<td>Etopophos® VePesid®</td>
<td><em>Podophyllum peltatum</em> (Plant)</td>
<td>Testicular and small cell lung cancer</td>
<td>Topoisomerase II inhibition</td>
<td>S and G2/M phase</td>
</tr>
<tr>
<td>Mitomycin C</td>
<td>Mutamycin®</td>
<td><em>Streptomyces lavendulae</em> (Microbe)</td>
<td>Gastric, colorectal, anal and lung cancer</td>
<td>DNA alkylatation and cross linking</td>
<td>Non-specific</td>
</tr>
<tr>
<td>Paclitaxel/Docetaxel</td>
<td>Taxol®</td>
<td><em>Taxus brevifolia</em> (Plant)</td>
<td>Ovary, breast, lung, bladder, and head and neck cancer</td>
<td>Promotion of microtubule stabilisation</td>
<td>G2/M phase</td>
</tr>
<tr>
<td>Topotecan/Irinotecan</td>
<td>Hycamtin®</td>
<td><em>Camptotheca acuminate</em> (Plant)</td>
<td>Ovarian, lung and paediatric cancer</td>
<td>Topoisomerase I inhibition</td>
<td>S and G2/M phase</td>
</tr>
<tr>
<td>Vinblastine</td>
<td>Velban® Velbe®</td>
<td><em>Catharanthus roseus</em> (Plant)</td>
<td>Bladder, kidney, lung, leukaemia, prostate and germcell ovarian cancer</td>
<td>Microtubule assembly inhibition</td>
<td>M phase</td>
</tr>
<tr>
<td>Vincristine</td>
<td>Oncovin® Vincrex®</td>
<td><em>Catharanthus roseus</em> (Plant)</td>
<td>Leukemia, lymphoma, neuroblastoma and rhabdomyosarcoma</td>
<td>Inhibition of tubulin polymerization</td>
<td>M phase</td>
</tr>
</tbody>
</table>
2.1.3 Targeting natural products

Drug discovery and clinical therapy are extremely important for the development of more effective new anticancer drugs as targeted therapeutics with tailored treatment strategies and regimens. Currently research is focused, due to progress in cancer biology, on cancer specific mechanisms and molecular targets corresponding to that (Altmann and Gertsch, 2007).

Natural products for cancer chemotherapy are often very potent but have limitations in terms of solubility in aqueous solvents and they show narrow therapeutic index and this caused the termination of a large number of pure natural products such as bruceantin and maytansin (isolated form *Maytenus serrata*) (Cragg and Newman, 2005). Alternatively these agents should be investigated and developed as potential “warheads”, by attaching these agents to monoclonal antibodies that are targeted specifically to epitopes on the tumours of interest (Sausville et al, 1999). With the emergence of novel technologies reviving interest in “old” agents could make it possible for them to be developed into effective drugs. Dedicated research over a period of 20-30 years period for clinical agents such as topotecan, paclitaxel (taxol), irinotecan and the camptothecin derivatives eventually led to the confirmation of their efficiency.

Revived interest was also found for ‘bruceantin’ isolated from *Brueca antidysenterica* J.F. Mill. (Simaroubaceae), Since it showed substantial activity against panels of leukaemia, lymphoma and myeloma cell lines, as well as in animal models bearing early and advanced stages of the same cancers, (Cragg and Newman, 2005). Previously, bruceantin showed activity in animal models. However, during its clinical trials no objective response was found. Further development therefore ended. Currently there is strong evidence supporting further development of bruceantin for the treatment of haematological malignancies. Its activity was linked with the down-regulation of (c-MYC), a key oncoprotein (Cragg and Newman, 2005).

Development of new derivatives from natural products with improved antiproliferative profiles and chemo preventive activities is essential, but it can only happen if their molecular mechanism of action, their effects on cellular signalling process is entirely understood as well as their structure-activity relationships (Kuo et al., 2005). Another vital clinical problem that needs to be tackled is drug resistance (Johnstone et al., 2002).
2.1.4 Rationale for studying anticancer botanicals

Revival is taking place in medicinal botanicals (including herbal remedies) as part of complementary medicine for disease prevention and therapy as conventional medications have high costs, side effects and therapeutic limitations (Park and Pezzuto, 2002). Enthusiasm and exceptional growing public interest for botanicals are not only found in the United States where about 40% of the Americans are using alternative medicine, but also in other parts of the world. Reduced risk of cancer was suggested by high consumption of fruits and vegetables in epidemiologic studies. Therefore, the great interests and enthusiasm in naturally occurring phytochemicals for cancer chemoprevention (Park and Pezzuto, 2002).

Whole botanicals (extracts) are seen as effective and safe to the general public, but investigative and conceptual scientific evidence is difficult to obtain. Extracts, herbal preparations or botanical medicine contain many compounds and pose significantly more conceptual challenges during research than that of a single compound, because they contains unknown components with unknown properties, the different components may act together as a barrier to the toxic effects of a single compound (buffer) and number of different compounds in combination may have synergistic activities (Vickers, 2002). They are also not subjected to the same regulatory standards than the other conventional medicine. Furthermore, possible drug interactions, recommended dosage and schedules create a lot of concern.

There are several reasons why whole botanical extracts, containing many unknown compounds, should be used or may benefit in anticancer treatment because there is the possibility is that whole botanical extracts can decrease the adverse effects as well as synergistic activity. There could also be the possibility of antagonistic activity due to multiple component interaction and competitive binding to common sites. Some have speculated that synergy results from the existence of “redundancy and back-up mechanisms found in the key regulatory and metabolic pathways of the cell” (Darzynkiewicz et al., 2000).

A number of different compounds in combination may have synergistic activity by targeting both primary and back-up mechanisms simultaneously. The use of whole plant botanicals or extracts could also reduce toxicity because of buffering taking place between the different constituents (Vickers and Zollman, 1999).
2.2 Targeting the Mitotic Spindle - A Review.

Mitosis is an ordered series of fundamentally mechanical events in which identical copies of the genome are moved to two discrete locations within the dividing cell (Figure 2.1). The chromosome segregation during mitosis occurs on a bipolar spindle that consists of dynamic structures, the microtubules, and of associated proteins, contributing to the assembly and functionality of the whole spindle complex. For decades the mitotic spindle was identified as an important target in cancer chemotherapy because of its crucial role in cell division.

![Figure 2.1 – Schematic illustration of the phases of mitosis.](image)

The mitotic spindle apparatus serves as the fundamental mechanical platform through which many of the processes described above occur. It is a self-organising molecular machine whose primary role is separation of the duplicated set of chromosomes to separate locations within the cell through transport to either end of its poles (Figure 2.1) (Wittmann et al, 2001). Half of a replicated chromosome must arrive at opposite spindle poles for accurate genetic
partition in the formation of two healthy daughter cells. Chromosomal segregation is achieved through two mechanisms, both of which are reliant on the microtubule scaffold of the spindle. Microtubules are rigid and polar polymers formed from α- and β-tubulin heterodimers which exhibit complex polymerisation dynamics during which they rapidly polymerise and depolymerise. The most prevalent expression of this behaviour in cells is termed dynamic instability (Jordan and Wilson, 2004). Associated with this activity is the hydrolysis of ATP by β-tubulin subunits to produce energy for mechanical work. The second way in which microtubules facilitate separation is by serving as tracks utilised by the mechanochemical proteins involved in mitosis. (Sharp et al, 2000).

**Mitotic spindle assembly**

Two motile processes are involved in the assembly and disassembly of microtubules and in segregation of chromosomes. The first is the polymerization and depolymerization of microtubules (Figure 2.2) physiologically regulated by a balance of microtubule stabilizing and destabilizing proteins, binding along the microtubules (Andersen, 2000).

**Figure 2.2: Structure and polarity patterns of microtubules in the metaphase spindle**

Microtubule assembly occurs at the fast growing plus end by tubulin polymerization and disassembly at the minus end by depolymerisation
Stabilizing proteins include the large group of the so-called microtubule-associated proteins (MAPs) (Mandelkow and Eva-maria, 1995). These MAPs are substrates of the cyclin-dependent kinase 1 (CDK1), and, depending on their phosphorylation state, they control the microtubule dynamic properties in the transition from the G2 to the M phase of the cell cycle. During mitosis an increased degree of phosphorylation reduces the affinity of the MAPs to microtubules, and thereby their promoting effect on tubulin-polymerization (Masson and Thomas, 1995; Ookata et al, 1995).

2.2.1 Limitations of microtubule based anti-mitotic agents

Taxanes and vinca alkaloids are highly cytotoxic agents and use in therapy gives rise to serious adverse effects (Jackson et al, 2007; Markman, 2003). As a key constituent of the cytoskeleton, microtubules possess a number of functional roles outside of mitosis, including in axonal transport in neurons (Morris and Hollenbeck, 1995). Resultantly, peripheral neuropathy is a common and severe toxicity amongst microtubule targeting drugs. Myelosuppresion, resulting from the cytotoxicity of anti-mitotics towards rapidly proliferating bone marrow cells is another regularly encountered dose limiting toxicity (DLT) (Gidding et al, 1999).

The other major concern with these regimens is resistance, which may be either acquired or innate. Certain cancers remain unresponsive to currently available anti-mitotic chemotherapies and determining the factors that govern tumour chemosensitivity is one of the most profoundly important currently unresolved questions in cancer chemotherapy. Acquired resistance can emerge through mutations affecting drug binding or by differing expression levels of β-tubulin isotypes (Kavallaris, 2010). High levels of expression of the βIII tubulin isotype are associated with more aggressive tumours and increased resistance to chemotherapy. Efficacy is also known to be reduced through increased expression of microtubule-associated proteins which regulate microtubule polymerisation dynamics, and can stabilise them against depolymerisation.

Mitosis is a highly regulated process during cell division. Anti mototic agents, such as the vinca alkaloid and taxanes, interfere with the polymerization-depolymerisation process of microtubule and lead to cell cycle arrest in mitosis. However, these agents target
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Microtubules and display several undesirable side effects in non-dividing cells, such as neurotoxicity (Wittmann et al., 2001). Thus, it is necessary to develop antimitotic drug that do not act on microtubules directly. Another approach is to target other protein, such as microtubule-associated protein or checkpoint proteins, the inhibition of which also leading to mitotic arrest and cell death (Heald, 2000). Because many of these protein are thought to have very specialized and specific functions as discrete phases of mitosis, inhibition may hopefully produce fewer side effects than known tubulin drugs.

2.2.2 Microtubule-based motor proteins

Two superfamilies of microtubule-based motor proteins have been discovered, the kinesins and the dyneins. Beside tubulin, the kinesins are the most prominent proteins contributing to the proper functionality of the mitotic spindle. The general structural features of the kinesin motors are similar throughout the whole protein superfamily (Vale and Fletterick, 1997).

Figure 2.3 – Domain organisation of conventional kinesin, a typical N-type kinesin.

The force-producing motor domain is divided into two major parts: one part is the globular catalytic core, which is conserved throughout the superfamily. The second part, the neck region, with a length of around 40 amino acids, is either adjacent at the N or C terminus of the catalytic core (Figure 2.3). Many Kinesin proteins contain a long α-helical coiled-coil domain, the “stalk”, which is often connected to an additional globular domain at its end.
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The latter domain, the “tail”, is thought to target the motor to a particular cargo within the cell.

The kinesins hydrolyze ATP and travel unidirectional along the microtubule surface. The N-terminal kinesins are plus end directed, and the C-terminal kinesins are minus end directed. In mitosis, the N-terminal kinesins produce an outward-directed force, whereas the C-terminal kinesins act vice versa. A further minus end directed force is exerted by the motor proteins of the dynein superfamily. During interphase and prophase, the duplicated centrosomes and slightly interdigitating microtubules between the two future poles of the spindle are formed. This initial spindle assembly is balanced by dynein on the actin cortex at the cell membrane pulling the poles apart (outward-directed force), and C-terminal kinesins in the spindle midzone pulling the poles together (inward-directed force). The C-terminal Kinesin force is limited due to the low extent of microtubule overlap in the midzone. However, with the continuous growth of the interdigitating microtubules, the kinesin force gradually increases until a steady state between the dynein and the kinesin forces is reached. After the degradation of the nuclear envelope in the prometaphase, another steady state is achieved. The length of the astral microtubules is decreased and the position of the bipolar spindle is determined and maintained by the action of the C-terminal kinesins and the bipolar kinesins (e.g. Eg5 kinesin). The bipolar kinesins are also located in the spindle midzone and generate an outward-directed force. The poles move further apart due to cortical dynein plus bipolar Kinesin activity, overwhelming the inwardly directed forces generated by the C-terminal Kinesin and tipping the balance of forces in the outward direction. At the onset of anaphase, the elongation of the spindle results in the “metaphase spindle steady state structure” with tension between the spindle poles, generated by antagonistic “inward” and “outward” forces. After passing the metaphase-anaphase checkpoint (mitotic spindle assembly checkpoint) the tension is released by inactivation of the C-terminal kinesins. Just before the disassembly of the spindle, the final steady state is achieved in the telophase.

It has been suggested that the expression of certain subtypes is restricted to proliferating tissues, and crucial for mitosis progression due to the cell cycle-dependent degradation of several kinesins (Funabiki and Murray, 2000; Hill et al, 2000). Furthermore, these mitotic kinesins have been shown to play essential roles during discrete phases of mitosis. Dysfunction of certain members of this protein family has been shown to result in mitotic
arrest. For example, the kinesin CENP-E has been shown to be an essential component of the mitotic spindle assembly checkpoint in vitro (Abrieu et al., 2000). It connects the checkpoint complex to free kinetochores of microtubule-attached chromosomes (Yao et al., 2000) and interacts with different kinetochore proteins. Playing a vital role in mitosis, this and other kinesins have been considered as potential new pharmacological targets for the treatment of malignancies.

**Table 2.2: Role of various mitotic kinesins in mitosis (Wood et al, 2001).**

<table>
<thead>
<tr>
<th>Mammalian Kinesin</th>
<th>Functional role</th>
<th>Experimental validation</th>
<th>Mitotic arrest</th>
</tr>
</thead>
<tbody>
<tr>
<td>Eg5</td>
<td>Spindle pole separation</td>
<td>Human cells</td>
<td>+</td>
</tr>
<tr>
<td>HSFT</td>
<td>Microtubule anchorage at the spindle poles</td>
<td>Human cells</td>
<td>-</td>
</tr>
<tr>
<td>MKLP1</td>
<td>Microtubule organization in metaphase spindle midzone Late mitotic spindle microtubule organization and cytokinesis</td>
<td>Human cells Drosophila, Caenorhabditis elegans</td>
<td>+</td>
</tr>
<tr>
<td>Kif4</td>
<td>Metaphase chromosome alignment</td>
<td>Xenopus egg extracts and embryos</td>
<td>n. d.</td>
</tr>
<tr>
<td>CENP-E</td>
<td>Metaphase chromosome alignment</td>
<td>Human cells</td>
<td>+</td>
</tr>
<tr>
<td>Kid</td>
<td>Metaphase chromosome alignment</td>
<td>Xenopus egg extracts</td>
<td>n. d.</td>
</tr>
<tr>
<td>MCAK</td>
<td>Anaphase chromosome alignment</td>
<td>Human cells</td>
<td>-</td>
</tr>
</tbody>
</table>

Dysfunction in kinesin causes mitotic arrest (+), dysfunction does not cause mitotic arrest (-); effect not determined (n. d.).

### 2.2.3 Kinesins in mitosis

The mitotic spindle apparatus serves as a suitable substrate for kinesin based motility events that lead to the generation of forces necessary for bipolar spindle formation, chromosome congression to the metaphase plate and segregation during anaphase, as well as cytokinesis. (Good et al, 2011) The establishment of a bipolar spindle requires the separation of the duplicated centrosomes through the sliding of overlapping antiparallel microtubules, a
process which requires the collaboration of different plus-end directed kinesins, such as Eg5 and Kif15/HKLP2, as well as the antagonistic action of minus-end directed motors including KifC1/HSET and dynein. (Tanenbaum and Medema, 2010) Chromosomal dynamics are also controlled by members of the kinesin-4 and kinesin-10 families termed chromokinesins: these associate to chromosome arms during mitosis, and contribute to the generation of forces, named polar ejection forces, that push the chromosome arms away from the poles and counter forces that drive chromosomes towards the poles. (Mazumdar and Misteli, 2005).

**Kinesin inhibitors for chemical biology and therapy**

Inhibitors of mitotic kinesins may act as biochemical tools through which the processes involved in mitosis may be understood further and to investigate potential pathways for treatment. (Bergnes et al, 2005). The synergistic activity of multiple antagonistic and complementary motor proteins, such as the kinesins described, is responsible for controlling the dynamic balance witnessed during spindle morphogenesis and subsequent chromosome movements. Interference with RNAi has suggested that at least twelve human kinesins are essential for the successful completion of cell division. (Zhu et al, 2005) It therefore follows that inhibition of these kinesins with specific small molecule inhibitors can elucidate the mechanisms underlying mitosis and their key regulators, and significantly may be used to disrupt mitosis for therapeutic purposes.

**Eg5 Kinesin inhibitors**

Sakowicz et al, 2004 screened a panel of compounds for selective inhibition of kinesin in *vitro* and identified the first kinesin inhibitor, adociasulfate-2, which competitively inhibits the interaction of the kinesin motor domain with microtubules. However, this first kinesin inhibitor did not show any selectivity between the three kinesin subtypes. Since only the kinesin Eg5 is known to cause mitotic arrest with the formation of a monopolar mitotic spindle, the monoaster spindle was used as an indicator in the search for specific Eg5 inhibitors. Using a mitotic spindle phenotype-based screening method, Mayer et al, 1999 identified monastrol as the first specific kinesin Eg5 inhibitor. This compound, a 4-aryl-3,4-dihydropyrimidine-2(1H)-thione, led to a reversible mitotic arrest by the formation of a monopolar spindle, and did not affect the transition from the G2 cell cycle phase to mitosis.
Moreover, monastrol did not inhibit the motor activity of conventional kinesin heavy chain, and therefore, had no effect on cellular processes involving other kinesins (Mayer et al, 1999). These results provide the first evidence that the concept of the specific Eg5 kinesin inhibition as a new approach in cancer chemotherapy is feasible. However, racemic monastrol and its eutomer, (S)-monastrol, were determined to be only moderately potent allosteric inhibitors of Eg5 (Gartner et al, 2005) with IC\textsubscript{50} values of 34 μM and 14 μM, respectively, determined in the microtubule-stimulated ATPase activity assay (Maliga et al, 2002). Therefore, new monastrol analogs were synthesized in order to obtain more potent Eg5 kinesin inhibitors. Hotha et al., 2003 reported on the tetrahydro-β-carboline compound HR22C16, which selectively inhibited Eg5 kinesin with an IC\textsubscript{50} value of 800 nM. A derivative of HR22C16 showed an IC\textsubscript{50} value of 90 nM, which is about one order of magnitude more potent than HR22C16. After the screening of a series of 60 β-carboline derivatives for Eg5 inhibitory activity, the most potent compound showed an IC\textsubscript{50} value of 650 nM (Sunder-Plassmann et al, 2005). Further lead structures are currently considered as potent and specific kinesin Eg5 inhibitors. Among them are terpendole E (IC\textsubscript{50} = 23 μM) (Nakazawa et al, 2003), CK0106023 with a Ki value of 12 nM Sakowicz et al, 2004, and KSP-IA, a compound derived from a dihydropyrazole lead structure (IC\textsubscript{50} = 10 nM (Tao et al, 2005). In a high-throughput screening effort, identified a series of tetrahydroisoquinolines as inhibitors of human Eg5 including a screening hit with an IC\textsubscript{50} value of 9.7 μM (ATPase assay) (Tarby et al, 2006). A medicinal chemistry optimization effort led to the identification of “compound 32a” as a potent Eg5 inhibitor with an IC\textsubscript{50} value of 104 nM.

2.2.4 Natural products as Kinesin inhibitors

Although most of the Eg5 inhibitors were synthesized in the laboratories, some natural compounds were reported to have potent Eg5 inhibitory activity. Terpendole E [a fungal indoloditerpene] was the first reported Eg5 inhibitor from natural origin (Motoyama et al, 2012). Originally, Ter E was extracted from the culture broth of a soilisolated fungus Albophoma yamanashiensis as an acyl-CoA:cholesterol acyltransferase inhibitor (ACAT). In 2003, its specific inhibition of Eg5 from microbial metabolites was reported. Terpendole E was found to inhibit chromosome segregation resulting in monopolar spindles in M phase, a picture specific for Eg5 inhibitors. Ter E was reported as inhibitor of both motor and
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microtubule-stimulated ATPase activities of human Eg5 (IC50 ¼ 23 mM). Ter E was essential for the Eg5 inhibitory activity since its epimerization or conversion to carbonyl derivatives abolished the Eg5 inhibitory activity. Gossypol is another natural Eg5 inhibitor. It is a small molecule isolated from cotton seeds. Preclinically, it showed potent anticancer activity especially against prostatic and mammary cancer cells. Gossypol displayed good clinical activity as mono and/or combined therapy in phase I/II trials for the treatment of prostate cancer and lung cancer. Gossypol exhibited axial chirality and existed as two enantiomers; gossypol more potent than racemic gossypol against breast cancer.