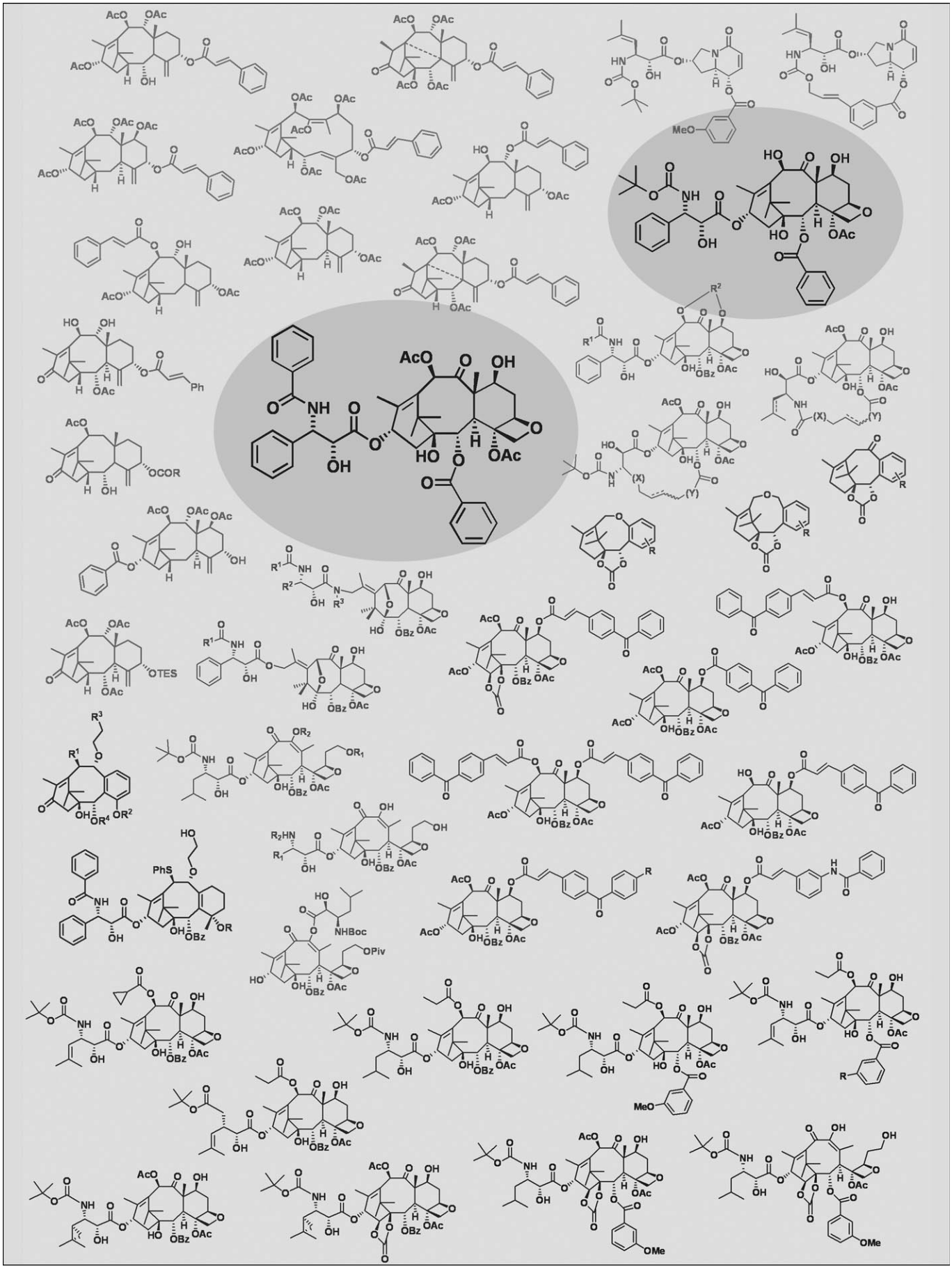

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Paclitaxel And Docetaxel Resistance: Molecular Mechanisms and Development of New Generation Taxanes

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Taxanes represent one of the most promising classes of anticancer agents. Unfortunately, their clinical success has been limited by the insurgence of cellular resistance, mainly mediated by the expression of the MDR phenotype or by microtubule alterations. However, the remarkable relevance of paclitaxel and docetaxel in clinical oncology stimulated intensive efforts in the last decade to identify new derivatives endowed with improved activities towards resistant tumor cells, resulting in a huge number of novel natural and synthetic taxanes. Among them, several structurally different derivatives were found to exhibit a promising behavior against the MDR phenotype in terms of either MDR inhibiting properties, or enhanced cytotoxicity compared to parental drugs, or both. On the other hand, only in more recent years have the first taxanes retaining activity against resistant cancer cells bearing alterations of the tubulin/microtubule system emerged. This review describes the main molecular mechanisms of resistance to paclitaxel and docetaxel identified so far, focusing on the advances achieved in the development of new taxanes potentially useful for the treatment of resistant tumors.

Introduction

Paclitaxel and docetaxel (**1** and **2** in Figure 1, respectively), progenitors of the family of taxanes, are well known anticancer drugs currently used in clinics for the treatment of several kinds of tumor, including ovarian, breast, head and neck, lung, and prostate cancer. These agents act as microtubule stabilizers and disrupt microtubule dynamics, thus inducing mitotic arrest and ultimately, cell death by apoptosis.[1]

Despite the relevant contribution of taxanes in ameliorating the quality of life and overall survival of cancer patients, the development of cellular resistance represents a serious limitation to their clinical use. The two main mechanisms involved in resistance to taxanes are the expression of the multidrug resistance (MDR) phenotype and the alterations of their cellular target, namely the tubulin/microtubule system.[2,3] Several less studied putative mechanisms of resistance, including alterations in the signaling pathways, altered regulation of the cell cycle and altered control of apoptosis and cell death signals, have also been described.[4] MDR is a term used to describe the ability of drug-resistant tumors to exhibit simultaneous resistance to a number of structurally and functionally unrelated chemotherapeutic agents.[2] The MDR phenotype is often mediated by the overexpression of drug efflux pumps, of which P-glycoprotein is the best known, that prevent the accumulation of the drugs within resistant cells. MDR was the first and most widely reported mechanism of resistance to taxanes; however, more recent studies described resistant tumor cells that neither overexpressed multidrug transporters nor showed a reduced drug accumulation, revealing that even changes of the microtubule structure or composition could lead to a reduced sensitivity of tumor cells to antimicrotubule agents through alterations of microtubule dynamic properties and/or of drug–target interactions.[3]

The clinical importance of paclitaxel and docetaxel in the treatment of solid tumors has stimulated intensive efforts to elucidate the molecular mechanisms of resistance to taxanes and to develop novel agents effective against resistant

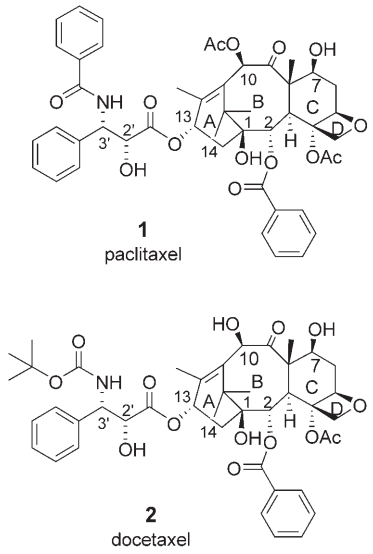

Figure 1. Structures of paclitaxel (**1**) and docetaxel (**2**).

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tumors.[5,6] Different approaches, such as identification of MDR-transporter inhibitors, synthesis, and evaluation of more active analogues, synthesis of conjugates or prodrugs as well as combined use with other drugs have been pursued to overcome taxane resistance.[7]

This review will focus on the role of natural and synthetic taxanes in overcoming paclitaxel and docetaxel resistance. Section 1 will deal with multidrug transporter-mediated mechanisms of resistance; the main transporters associated with MDR, and the possible strategies for circumventing them will be briefly outlined, followed by a review on the development of natural and synthetic taxanes potentially useful for the treatment of MDR tumors, owing to their capability to either inhibit MDR efflux pumps or to bypass their action. Novel antimicrotubule taxanes which showed promising activity in the treatment of drug-resistant cells and currently undergoing clinical evaluation will also be reported. Section 2 will deal with mechanisms of resistance involving alterations of the biological target of taxanes; the most recent advances in identifying and elucidating the clinical role of distinct mechanisms will be discussed, and the taxanes reported to retain antimitotic activity against cancer cells bearing changes in the tubulin/microtubule system will be described.

1. Taxane resistance associated with multidrug transporters

The most extensively studied mechanism of resistance to taxanes is the overexpression of P-glycoprotein and other multidrug transporters: these are membrane proteins belonging to the ATP-binding cassette (ABC) family of transporters,[8,9] and act as efflux pumps which extrude a large number of structurally diverse, mainly hydrophobic compounds from cells, thus keeping intracellular drug concentration below a cell-killing threshold and inducing cross-resistance to several chemically unrelated compounds. ABC transporters are widely distributed in normal tissues; although their exact physiological role is still to be fully elucidated, they are thought to prevent cytotoxic compounds in the environment and diet from entering the body and remove them by excretion into the bile and urine.

1.1. Multidrug transporters

The best known and well-studied multidrug transporters are P-glycoprotein (P-gp), encoded by the *mdr*1 gene,[10] multidrug resistance protein 1 (MRP1), encoded by the *mrp*1 gene,[11] and

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breast cancer resistance protein (BCRP), encoded by the *mxr* gene.[12]

Selection of cancer cell lines with paclitaxel or other anticancer drugs, frequently results in MDR mediated by increased expression of P-gp. P-gp is an ATP-dependent broad-spectrum multidrug efflux pump, consisting of two homologous halves joined by a linker region (Figure 2a). Each half begins with a

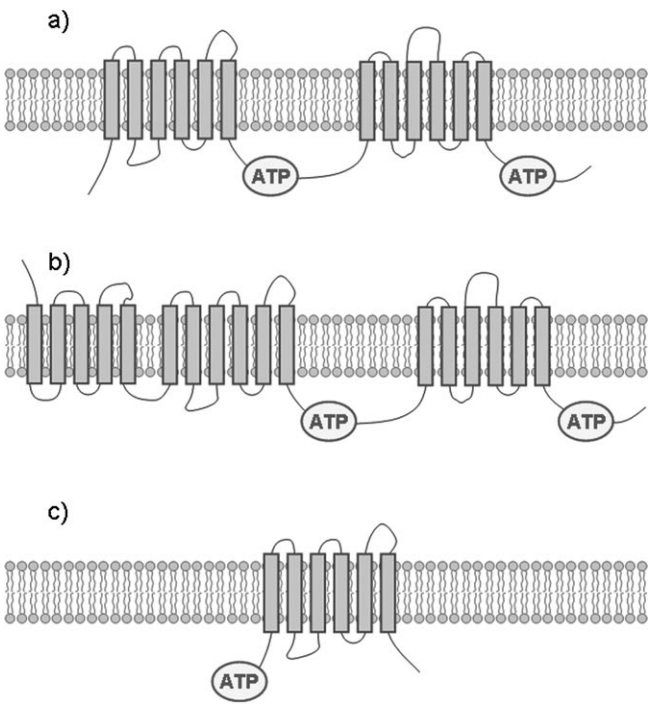

Figure 2. Structures of multidrug transporters: domain arrangement in a) P-gp, b) MRP1, and c) BCRP. Circles represent the ATP-binding domains; cylinders represent the segments of transmembrane domains.

transmembrane domain, (which binds hydrophobic drug substrates) containing six transmembrane segments, followed by a hydrophilic region containing the ATP binding site.[13] Increased levels of P-gp are common in some tumor types and have been frequently associated with paclitaxel resistance: evaluation of *mdr*1 gene expression in a NCI 60 cell line anticancer drug-screening panel demonstrated a correlation of *mdr*1 expression with the sensitivity profile of paclitaxel,[14] and several authors have detected increased levels of either *mdr*1 mRNA or P-gp itself in paclitaxel-resistant cell lines.[15,16] Nevertheless, much remains to be learned about the role of the mechanisms of resistance mediated by P-gp and other ABC-transporters in different human tumors and their relevance for patients receiving a taxane-based chemotherapy.[8,17,18] Besides mediating taxane resistance in tumor cells, P-gp may also play a significant role in modulating taxane absorption and tissue distribution; in this regard, the high expression of P-gp in the intestinal mucosa has been shown to strongly limit the oral bioavailability of paclitaxel[19] and the marginal efficacy of the drug against primary brain tumors is consistent with its inability to cross the intact blood-brain barrier, where P-gp is highly expressed.[20]

MRP1 is the most studied protein of the MRP family, which comprises six other characterized members (MRP2, MRP3, MRP4, MRP5, MRP6, MRP7).[11,21] Like P-gp, MRP1 has a core structure consisting of two membrane spanning domains, each of them being followed by an ATP-binding domain, but it also contains a third N-terminal transmembrane domain consisting of five transmembrane segments (Figure 2b). Whereas P-gp targets and transports hydrophobic drugs, MRP protein recognizes hydrophilic molecules and organic anions; it also transports neutral drugs conjugated with glutathione, glucuronide, or sulfate, and some anticancer agents by co-transport with glutathione. However, unlike P-gp, to date MRP seems to play a marginal role in resistance to taxanes.[22]

As represented in Figure 2c, BCRP consists of a N-terminal ATP-binding site and six transmembrane segments; it is a half-transporter likely to homodimerize or heterodimerize to function.[23] It was initially isolated from breast cancer cell lines which demonstrated doxorubicin resistance. Although BCRP does not confer resistance to taxanes, noncytotoxic synthetic taxanes have been shown to be able to modulate BCRP-mediated drug efflux.

1.2. Overcoming transport-based resistance: general overview

As resistance to taxanes induced by P-gp and related MDR efflux pumps is one of the main obstacles to successful chemotherapy of cancer, several strategies for blocking the extrusion of drugs and circumventing cross-resistance mediated by these transporters have been proposed (reviewed in refs. [24] and [25]), including the inhibition of transporters (engage), the use of cytotoxic agents that are not substrates for MDR proteins and can therefore bypass the efflux from the cell (evade), and approaches that take advantage of the collateral sensitivity of MDR cells (exploit) (Figure 3).

Several compounds have been shown to inhibit the drug efflux function of P-gp and therefore reverse cellular resistance (engage strategy, Figure 3b). Such MDR modulators (or MDR reversal agents) can be co-administered together with cytotoxic agents and belong to a number of different chemical classes,[26] which also include taxanes, as described in sections 1.3.1 and 1.4.1. Calcium channel blockers, such as verapamil, were the first agents demonstrated to be able to reverse MDR[27] and constituted the first generation of MDR modulators. The unique property shared by most first generation MDR modulators, typically therapeutics agents already known or used for other purposes, was their capability to reverse MDR at concentrations much higher than those required for their individual therapeutic activity. Further investigations led to second and third generation modulators, which have been developed through structure–activity relationships and combinatorial chemistry approaches and are active at concentrations of nanomolar range.[26] However, despite the promising advances in preclinical models, to date clinical studies on MDR modulators have met with limited success.[25]

P-gp mediated MDR can also be reversed by hydrophobic peptides which correspond to the transmembrane segments

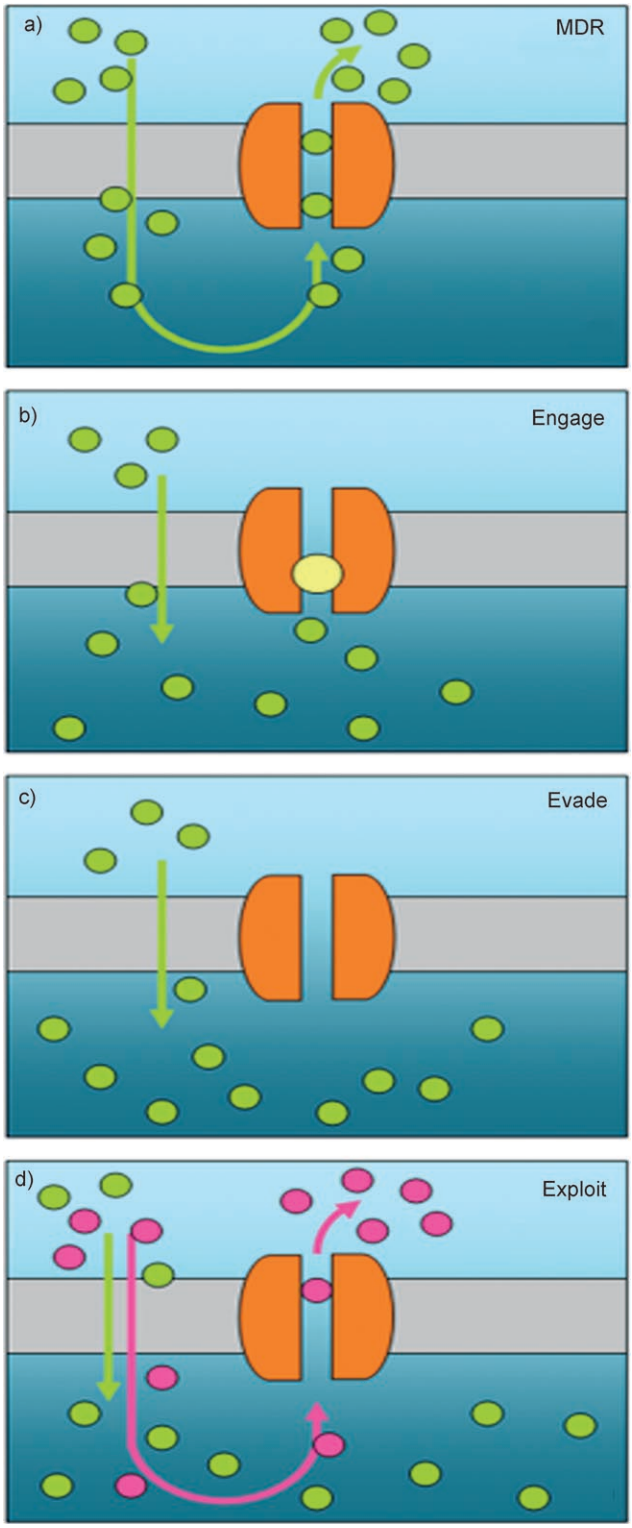

Figure 3. Possible strategies for overcoming drug resistance mediated by multidrug transporters. a) Multidrug transporters pump out cytotoxic drugs (green circles) from MDR cancer cells; b) Reversal agents (yellow circle) block the efflux pumps, preventing cross-resistant cells from extruding the anticancer drugs; c) Cytotoxic agents that are not substrates for multidrug transporters are not extruded from resistant cells; d) The cytoprotective agent (magenta circles), but not the cytotoxic drug, is pumped out from resistant cancer cells; only in this case MDR cancer cells can be selectively killed, since normal cells, which do not extrude the protector molecule, remain unharmed.

of P-gp and interfere with the proper assembly and functioning of the protein. Consistent with this idea, newly synthesized specific peptide inhibitors of P-gp have been recently shown to sensitize resistant cancer cells to chemotherapic agents, thus appearing to be a promising class of noncytotoxic drug resistance inhibitors.[28]

Among the strategies aimed at inhibiting the activity of MDR transporters (still in the engage field), research has recently shifted to the modulation of P-gp expression, either by blocking the expression of *mdr*1 mRNA through antisense oligonucleotides[29] or hammered ribozymes,[30] or by preventing the P-gp biosynthesis using chemical compounds.[31,32]

Another approach to overcome resistance mediated by ABC-transporters is based on the use of drugs which are not substrates for MDR proteins (evade strategy, Figure 3c), such as cyclophosphamide, cisplatin, and epothilones.[33] The latter are novel tubulin targeting anticancer agents that are not recognized by P-gp, thus providing proof that new classes of antitumor drugs not interacting with MDR proteins can be developed to improve the response to therapy. Furthermore, it has been demonstrated that chemical modifications of paclitaxel and docetaxel, MDR inducing compounds, can favorably result in active, but not transported, second generation taxanes, which will be described in section 1.4.2.

The exploit approach is based on the idea that drug efflux pumps can be exploited to selectively kill resistant cancer cells, while sparing sensitive normal cells; two main strategies have been proposed to date to take advantage of multidrug transporter overexpression in cancer cells. The first one involves the co-administration of a cytoprotective (antiapoptotic or cytostatic) agent, which is a substrate for efflux pumps, together with a cytotoxic agent which is not recognized by multidrug transporters: in the presence of a protective agent, normal cells remain unharmed, whereas resistant cells, which pump out the protecting agent, do succumb to cytotoxic therapy (Figure 3d).[34,35] The alternative strategy involves the use of anti-P-gp antibodies to destroy cells expressing P-gp, again resulting in selective killing of drug-resistant cells.[36]

1.3. Natural taxanes in overcoming transport-based resistance

Since the discovery of the promising anticancer activity of paclitaxel and some related compounds, chemical studies on constituents of different yew trees have resulted in the isolation of a large number of new natural taxanes. During the last two decades, approximately 120 taxanes with different skeletons, containing 5/7/6-, 6/10/6-, 6/8/6-, or 6/12-membered ring systems, have been isolated from the Japanese yew, *Taxus cuspidata*. Interestingly, some of these agents have been shown to reduce Ca^{2+}-induced depolymerization of microtubules, to increase cellular accumulation of vincristine in MDR tumor cells, and to exert significant cytotoxic activity. The structures, the biological activities, and the chemistry of taxanes isolated from *T. cuspidata* have been recently reviewed by Shigemori and Kobayashi.[37]

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