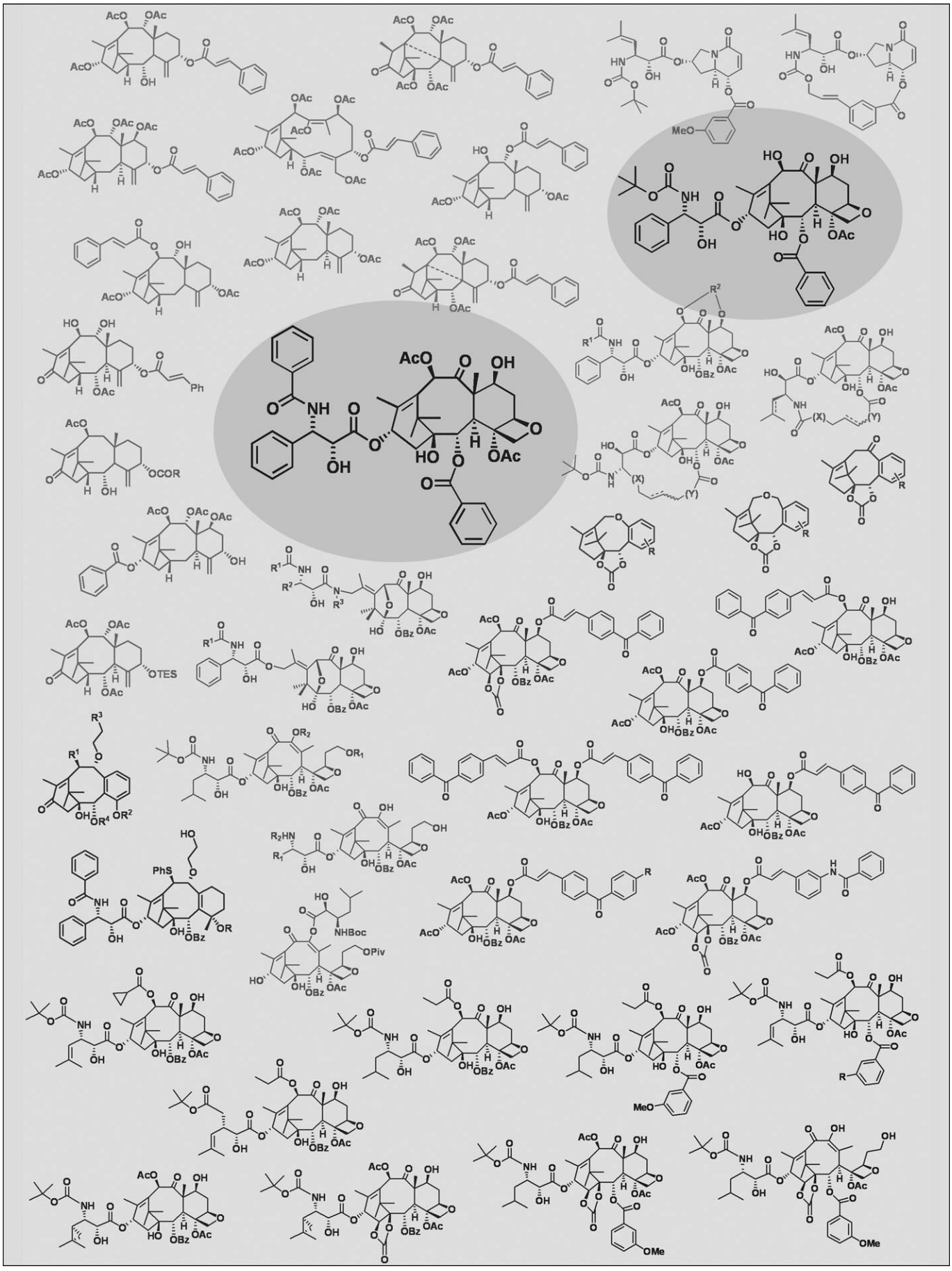

 DOI: 10.1002/cmdc.200600308

DOI: 10.1002/cmdc.200600308

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

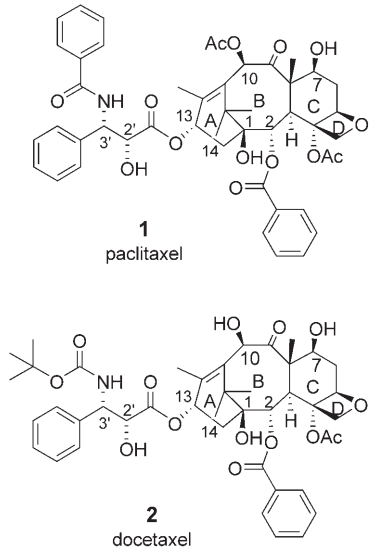

Figure 1. Structures of paclitaxel (**1**) and docetaxel (**2**).

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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

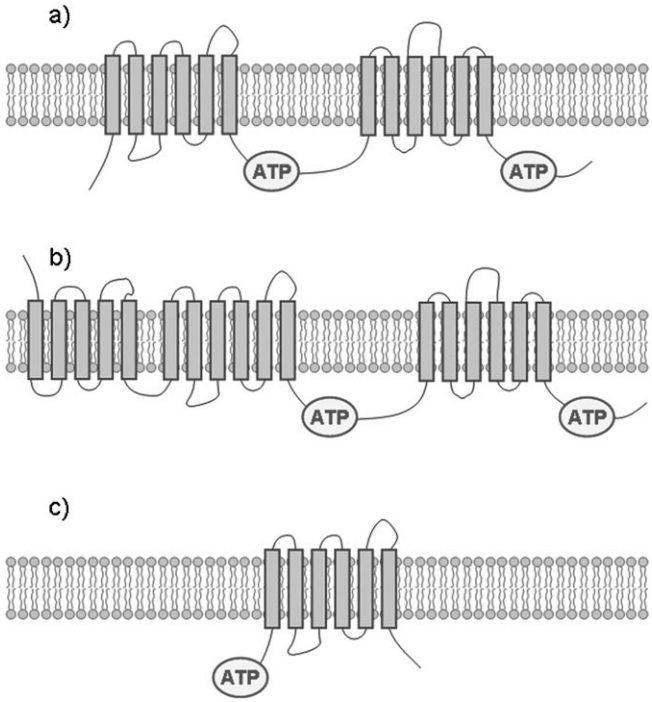


Figure 2. Structures of multidrug transporters: domain arrangement in a) P-gp, b) MRP1, and c) BCRP. Circles represent the ATP-binding domains; cylinders represent the segments of transmembrane domains.

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

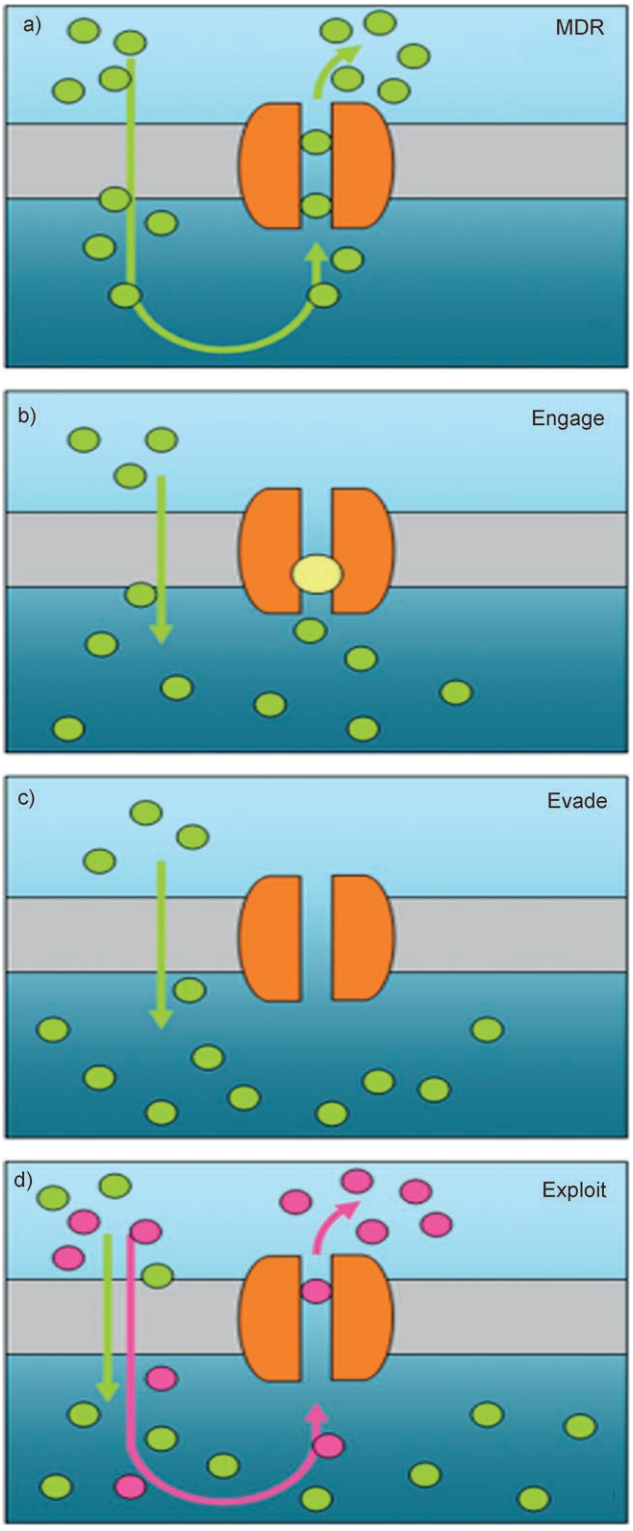


Figure 3. Possible strategies for overcoming drug resistance mediated by multidrug transporters. a) Multidrug transporters pump out cytotoxic drugs (green circles) from MDR cancer cells; b) Reversal agents (yellow circle) block the efflux pumps, preventing cross-resistant cells from extruding the anticancer 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]

1.3.1. Natural taxanes as MDR reversal agents

As anticipated, the effects of the above mentioned natural taxanes on the cellular accumulation of the antitumor drug vincristine (a P-gp substrate) in MDR human ovarian 2780AD cancer cells were examined, and the more promising compounds are reported in Figure 4: taxinine NN-1 (taxezopidine G, **3**) showed the strongest activity in terms of vincristine accumulation in MDR tumor cell, with a value of 323% of verapamil. Likewise, cyclotaxinine NN-2 (**4**) (204%),[38] 2-deacetoxytaxinine (**5**) (108%), taxuspine X (**6**) (105%), and taxuspine C (taxinine, **7**) (104%)[39] increased the vincristine accumulation more or as potently as verapamil. Moreover, taxuspine C was shown to reduce the binding of [^{3}H]-azidopine (a P-gp photoreactive substrate) to P-gp in the adriamycin-resistant human leukemia K562/ADM cell line more potently than verapamil,[40] and to completely reverse the resistance to colchicine, vincristine, and paclitaxel in human epidermoid carcinoma KB-C2 cells, which overexpress P-gp;[41] when co-administered with vincristine, taxuspine C increased the life span of mice bearing the vincristine-resistant leukaemia cells P388/VCR.[42]

Plant cell cultures of *Taxus* species have always been considered a promising approach to obtain paclitaxel and related taxanes in good amounts. In confirmation of that, Tsuruo and coworkers[43] reported the isolation of taxinine NN-11 (**8**) from callus culture of *T. cuspidata* cultivated on a modified Gamborg's B5 medium after stimulation with methyl jasmonate. Taxinine NN-11, whose structure is reported in Figure 4, exhibited about twofold higher activity than verapamil towards vincristine accumulation in the MDR 2780AD cell line. Further chemical investigation on the callus cultures of *T. cuspidata* led to the isolation of the new taxane **9** (Figure 4), which is the 9,10-isomer of taxinine NN-11 and showed 67 to 92% activity of verapamil at different concentrations on the cellular accumulation of calcein (another P-gp substrate) in 2780AD cells.[44] Analogously, taxusin (**10**, Figure 4) was isolated in the course of investigations on secondary metabolites and production of useful natural product in the dark brown callus culture of *T. cuspidata* incubated under light irradiation; this taxane exhibited stronger MDR reverse activity than verapamil, still towards 2780AD tumor cells.[45]

Taken together, these results demonstrate that natural taxanes could be good inhibitors of P-gp and useful agents in overcoming MDR, thus stimulating continued efforts in searching for new natural or synthetic paclitaxel derivatives endowed with improved MDR reversal activity.

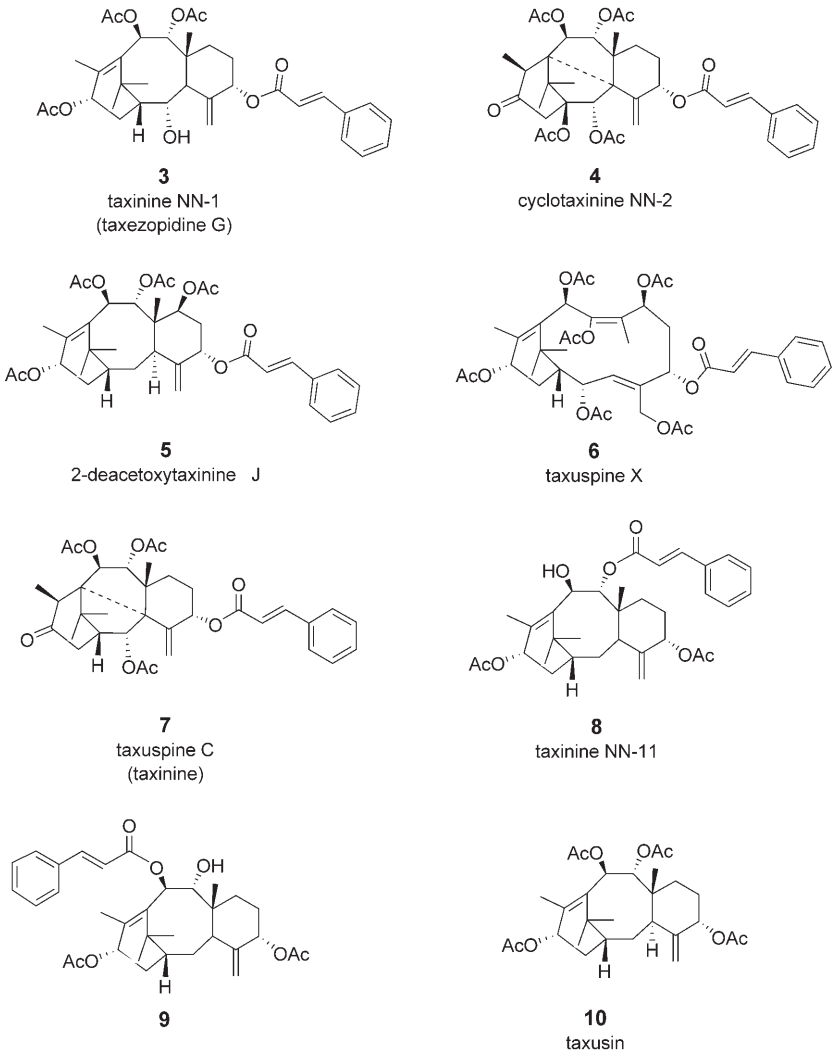


Figure 4. Taxanes from *T. Cuspidata* endowed with inhibitory activity towards the drug efflux function of P-gp in MDR cells.

1.3.2. Anticancer activity of natural taxanes

The cytotoxic activity of natural taxanes isolated from *T. cuspidata* (mentioned above), *T. yunnanensis*, and *T. chinensis*, has been examined against murine leukemia L1210 cells and human epidermoid carcinoma KB cells.[46] Some of them, including both paclitaxel-like and nonpaclitaxel-like compounds, exhibited a strong inhibitory activity against Ca^{2+}-induced depolymerization of microtubules, comparable to paclitaxel; however, despite the good activity against drug-sensitive cancer cells, natural taxanes evaluated so far have been reported to be ineffective against MDR cell lines, suggesting that, just like paclitaxel, these agents are substrates for multidrug transporters and are thereby extruded from resistant cells.

1.4. Synthetic taxanes in overcoming transport-based resistance

Significant results have been obtained in the synthesis of new generation taxanes: extensive SAR studies have led to the development of highly efficient taxane-based MDR reversal agents (TRAs) as well as cytotoxic taxanes endowed with higher potencies in comparison with paclitaxel on resistant cells, owing to their ability to evade the MDR transporters (second generation taxanes). It is noteworthy that some of these newly synthesized taxanes, able to overcome MDR, are now in different phases of clinical development.

1.4.1. Synthetic taxanes as MDR reversal agents

The use of noncytotoxic chemosensitizer taxanes (MDR reversal agents) able to block the binding site of anticancer drugs on P-gp and sister proteins, thus preventing their exclusion from the cells, has received considerable attention as a reliable strategy to inhibit the activity of multidrug transporters.

On the basis of the interesting activity of natural taxinines described above as P-gp inhibitors, the three novel taxinine analogues **11**–**13** (Figure 5) were prepared from sinenxan A (a readily available biosynthetic taxane),[47] and tested for their activity as MDR reversal agents in comparison with verapamil.[48] In vitro assays revealed for all the three compounds an interesting MDR reversal activity on KB/V cells, a MDR subline of human epidermoid cancer cells KB overexpressing P-gp; compound **12**, in particular, was shown to be more potent than verapamil. Further in vivo studies on vincristine-resistant KB/V tumor xenografts showed that **12** in combination with vincristine significantly inhibited the tumor growth, whereas treatment with vincristine or **12** alone did not result in growth inhibition.

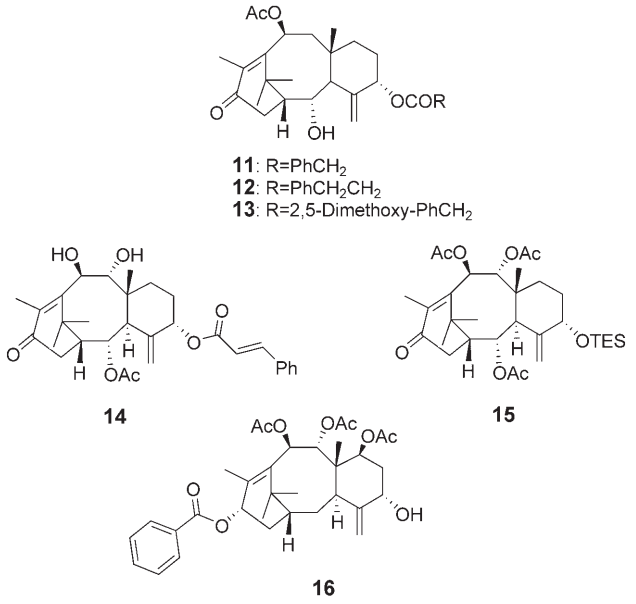


Figure 5. Examples of taxinine derivatives with MDR reversal activity.

A series of taxuspine C analogues were synthesized by Tsuruo and Kobayashi and their effect on the cellular accumulation of vincristine in MDR 2780AD cancer cells was examined.[49] Taxinine derivatives containing a cinnamoyloxy, a benzoyloxy, a TES, or a BOM group at C2, C5, or C13 were found to significantly increase the cellular accumulation of vincristine in MDR cells, suggesting that taxinine analogues could be good modifiers of MDR in resistant tumor cells; the most interesting compounds, **14** and **15** (Figure 5), exhibited higher potency than verapamil.

Very recently our group described the synthesis of a series of 2-deacetoxytaxinine J (**5**) derivatives and their evaluation as MDR reversal agents on the drug-resistant human breast cancer cell line MCF7-R, overexpressing P-gp; the most interesting results in terms of paclitaxel accumulation were achieved with taxinine **16** (Figure 5), bearing a benzoyl group at C13 position.[50]

A wide set of TRAs based on the 10-deacetylbaccatin III (DAB) and 14β-hydroxy-10-deacetylbaccatin III (14-OH-DAB) skeleton has been developed during the years and their structure–activity relationships have been recently reviewed by Ojima and co-workers.[51] DAB and 14-OH-DAB (**17** and **18** in Figure 6, respectively), even though noncytotoxic by themselves, provide the crucial components of paclitaxel and possess several hydroxy groups that can be easily modified with hydrophobic side chains by esterification. In addition, 14β-OH-DAB, isolated from the needles of the Himalayan yew tree (*T. wallichiana Zucc.*),[52] has substantially better water solubility than DAB due to the presence of an extra hydroxy group at the C14 position; taxanes derived from 14β-OH-DAB are therefore expected to have improved water solubility, bioavailability, and reduced hydrophobicity-related loss of efficacy.

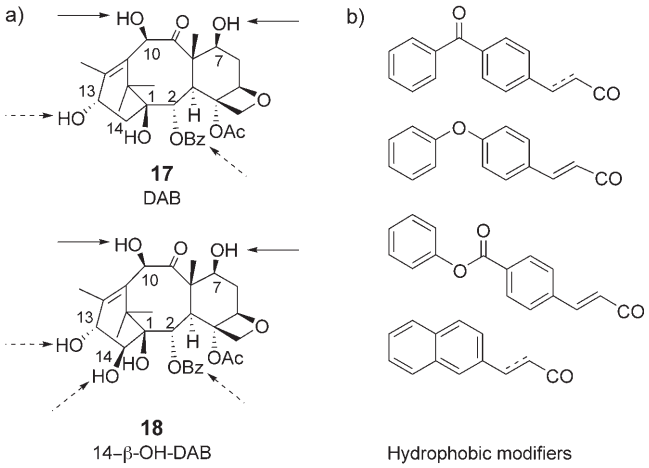


Figure 6. Design of TRAs by insertion of hydrophobic substituents at different positions of DAB and 14β-OH-DAB core: a) solid arrows indicate the most suitable positions for enhancing the MDR reversal activity; b) hydrophobic modifiers which gave the most interesting results in terms of MDR modulation.

The extensive SAR studies of TRAs focused on two aspects, namely the identification of both the structural requirements and the most suitable position for a potential pendant group.

Preliminary SAR studies on structurally different classes of MDR reversal agents pointed out the importance of the presence of a hydrophobic, conjugated, planar ring.[53] Accordingly, benzophenone, naphthalene-containing carboxylic acids, and other related hydrophobic groups have been chosen to modify the hydroxy groups at C2, C7, C10, and C13 positions of either DAB or 14β-OH-DAB. Among the hydrophobic pendant groups designed and examined, those consisting of two aromatic rings, spaced by a 1- or 2-atom linker and bearing a carbonyl or ether group, were identified as the most effective units (Figure 6).[51]

Over the years, several libraries of novel TRAs have been designed, synthesized, and evaluated for their modulating capability against P-gp, overexpressed in drug-resistant cancer cell lines MCF7-R and MDA-435/LCC6-MDR.[51] The results of these studies clearly indicated that modifications at the C7 position can result in strong MDR reversal activity and benzophenone and naphthalene appeared to be the most appropriate pendant groups. Even modification of the C10 hydroxy group with a benzophenone side chain resulted in a very good reversal activity, whereas the attachment of a hydrophobic side chain containing a diphenyl ether, a diphenyl thioether, a benzamide, and a benzoate did cause a significant loss of activity. The effects of the simultaneous introduction of two hydrophobic side chains at both the C7 and C10 positions on the reversal activity are more complicated. Modification at both C7 and C10 positions with benzophenone proved to be very favorable; replacement of the C10 benzophenone with either a methyl formate or a propanoyl group was well tolerated, whereas replacement with larger aromatic substituents or replacement of C7 benzophenone with naphthalene resulted in significant loss of activity. Modifications at the C2 or C13 position gave poor results. These findings strongly corroborated the idea that hydrophobicity is not the only feature necessary for an efficient MDR reversal activity, and that there is a specific binding site for TRAs on P-gp with rather strict steric/shape requirements.

When TRAs **19**–**22** (Figure 7) were co-administered at 1.0 μM, paclitaxel recovered 95–99.8% of its efficacy against the cross-resistant cancer cells MCF7-R and MDA-435/LCC6-MDR. Furthermore, two of these TRAs, namely **19** and **22**, exhibited high MDR reversal activity even at lower concentrations, together with a much higher activity than verapamil and slightly better activity than cyclosporine A.[54] To prove the mechanism of action for these agents, the effects of **22** on the paclitaxel uptake by the drug-resistant cancer cells MDA-435/LCC6-MDR in the presence and absence the taxane were investigated, demonstrating that **22** blocked the P-gp efflux mechanism, thus the anticancer drug was not extruded from drug-resistant cancer cells and exerted its chemotherapeutic effect.

The agents described so far, similar to most of the compounds belonging to the category of TRAs, are noncytotoxic up to the solubility limit (approximately 30 μM), therefore they have excellent therapeutic indexes. Finally, it is worth noting that neither DAB, 14β-OH-DAB, nor the hydrophobic modifiers showed any MDR reversal activity at all by themselves, and

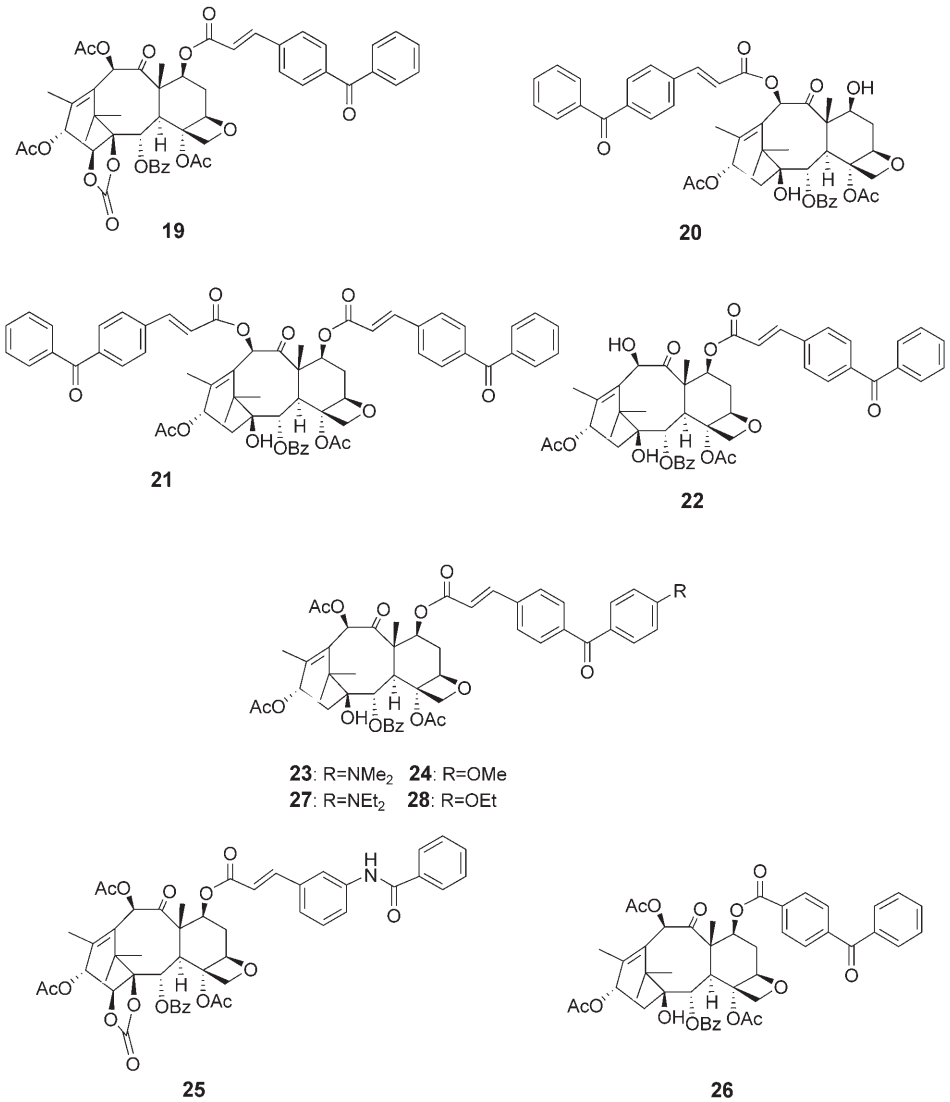


Figure 7. Examples of TRAs. Worthy of note, TRAs **23**–**28** are able to modulate the P-gp, MRP1 and BCRP efflux pumps.

therefore the MDR reversal activity was unique to the combination of their structures.[54]

The majority of initial studies concerning MDR reversal agents have only focused on modulators of P-gp protein. However, the true measure of the efficacy of a MDR reversal agent is represented by its ability to inhibit the drug efflux mediated by a broad spectrum of ABC transporters. On the basis of such a remark, among the large number of synthesized TRAs the best twenty compounds in terms of MDR reversal activity in combination with paclitaxel against the MDA435/LCC6-MDR cell line, overexpressing P-gp, were chosen to assess their capability to also block the MRP1 and BCRP efflux pumps. Accordingly, the efflux of mitoxantrone (which was demonstrated to be a substrate for P-gp, MRP1, and BCRP transporters)[55] was evaluated on drug-resistant human myelogenous leukemia and myeloma cell lines overexpressing MRP1 (HL60-ADR), P-gp (8226-Dox6), and BCRP (8226-MR20). Interestingly, the four agents **23**–**26**, plus the newly synthesized **27** and **28** (Figure 7) provided very good results, being able to strongly modulate not only P-gp, but also the other MDR-associated ABC transporters.[51,56]

In the course of studies towards new taxanes to be employed in overcoming transport-mediated MDR resistance, modifications of the taxane skeleton led to the C-aromatic taxanes, evaluated as MDR reversal agents by Tsuruo and coworkers.[57] Starting from the C-aromatic taxanes **29a** and **29b**, derivatives **29c**–**i** (Figure 8) were designed taking into account that the hydrophobicity of a molecule appeared to be important for P-gp affinity, as aromatic functional groups were incorporated into most of the known active compounds. Accordingly, a benzoyl group was chosen and linked to the hydroxy groups of **29a** and **29b** in most of the synthesized derivatives. The MDR reversal activity of the newly synthesized C-aromatic taxanes **29c**–**i** was then evaluated as an enhancing effect of vincristine accumulation in ovarian MDR cancer cells 2780AD, using verapamil as a positive control. The intermediate **29a** exhibited weak activity compared to verapamil, and most of the functional group transformations (**29c**–**e**) proved ineffective. However, a significant enhancement of the activity was observed for the benzoate derivatives, as the monobenzoate **29g** and especially the C2-benzoate **29h** exhibited the same potency as verapamil. Additional incorporation of a benzoyl group in **29f** and **29i** was again ineffective in enhancing activity. These results indicated that an aromatic functional group on the B-ring may play an important role in the interaction with P-gp, though it was not clear why the benzoyl group maintained its efficacy in different positions, as observed for derivatives **29g** and **29h**.

With the intriguing purpose of discovering compounds endowed with both MDR reversal and antitumor activity, the same authors also described the synthesis and the biological evaluation of taxanes **30** and **31** (Figure 8).[58] The taxane **30** proved to have no effect on vincristine accumulation in the cells, whereas **31** showed some activity, although slightly lower than verapamil. However, with regard to the antitumor activity, these compounds proved to be far less potent than paclitaxel, and because of their low activity they were not evaluated against drug-resistant cell lines expressing the MDR phenotype.

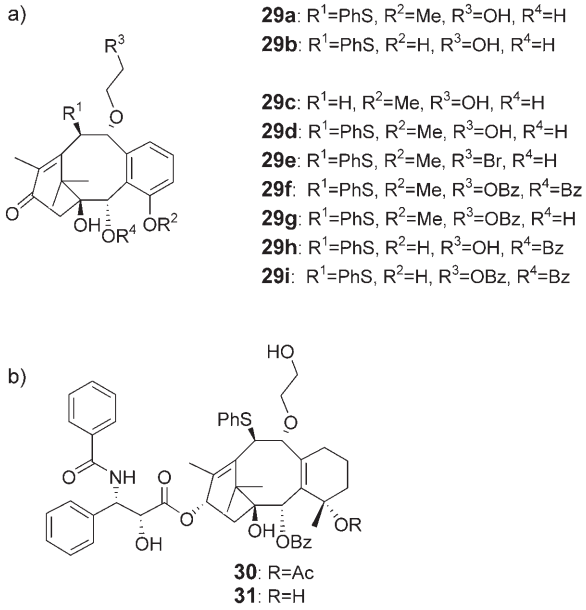


Figure 8. a) C-Aromatic taxanes as MDR reversal agents; b) first attempts to identify taxanes endowed with both MDR reversal and cytotoxic activity.

Among the studies concerning the synthesis of taxanes with P-gp affinity, aimed at engaging transport-based resistance, Takahashi and co-workers recently reported the synthesis and the biological evaluation of novel compounds with different 3D templates based on a taxane skeleton.[59] The three investigated core templates (**32**, **33**, and **34** in Figure 9) were characterized by different sizes of the B-ring as well as different functional groups attached to the B-ring itself. Conformational analysis of the three sets of compounds **a**--**c** suggested that all of them are likely to possess the endo conformation as in paclitaxel and that the O-functional group at the C4 or C5 position in series **b** and **c** is located at the same position as the C4 acetoxy group in paclitaxel.

Measurement of P-gp affinity for these compounds bearing a 3D core template of nonpaclitaxel type indicated a significant difference between OMOM substitution at the C4 position, the C5 position, and the unsubstituted analogue: in particular, the introduction of the O-functional group, especially at

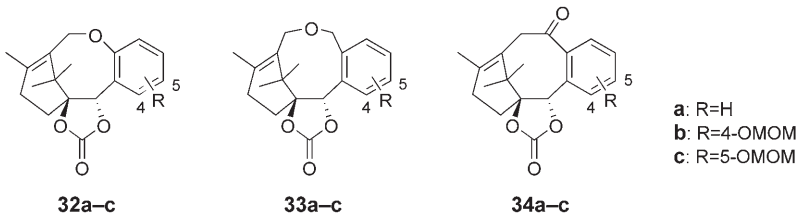
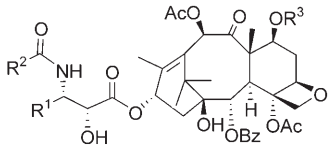


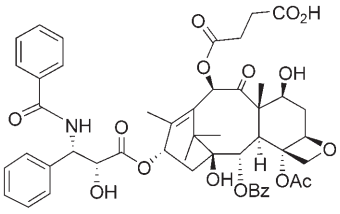
Figure 9. MDR reversal agents derived from different 3D templates based on a taxane skeleton.

the C5 position, was shown to enhance the P-gp affinity, although this value was lower than that of paclitaxel for all derivatives. Further analysis on human epidermoid carcinoma MDR KB-G2 and KB-3-1 cell lines revealed that all the compounds, except **33 b**, were not endowed with detectable cytotoxicity. Finally, when the MDR reversal activity of **33 c** (which exhibited the highest affinity to P-gp) was evaluated, it was demonstrated that this compound was able to sensitize MDR KB-G2 cells to paclitaxel in a dose-dependent manner.



35

R^1=Ph, 2-furyl, *i*butenyl
R^2=Ph, *t*butoxy
R^3=CH_2SCH_3, MOM, $CH_2O(CH_2)_2OH$, Me



36

Figure 10. C7 (general structure and possible substituents for each position) and C10 modified taxanes able to overcome MDR.

1.4.2. Cytotoxic synthetic taxanes overcoming transport-based resistance

During the search for novel taxanes exhibiting an improved activity profile against MDR tumor cells, appropriate modifications at the C2, C7, C10, and C3′ positions of paclitaxel and docetaxel led to the second generation taxane anticancer agents. These taxanes retained the microtubule stabilizing and cytotoxic properties of the parent compounds, showed increased or comparable potency against drug-sensitive human cancer cell lines, and most importantly, exhibited significant activity towards drug-resistant cell lines expressing the MDR phenotype, because of the low sensitivity to multidrug transporter-mediated efflux from cells.[7] Disruption of binding to MDR transporters, resulting in reduced efflux from cells, is a plausible mechanism to achieve activity in tumors that are unresponsive to paclitaxel treatment, and it was proposed that several modified taxanes able to overcome MDR were not good substrates for P-gp and other multidrug transporters. In this regard, changes in the C7 to C10 region of the molecules seemed to play a major role in impairing the interactions with P-gp, and the proximity of the C7 to the P-gp binding site of paclitaxel has been confirmed by photoaffinity studies.[60] However, more recently some second generation taxanes have been demonstrated to be endowed with cytotoxic and also MDR reversal properties, thus providing a new alternative mechanism to avoid extrusion from MDR cancer cells. In fact, such taxanes would act as self-modulating agents able to block their own efflux from multidrug transporters overexpressing cells, thereby retaining their anticancer activity against drug-resistant tumors.

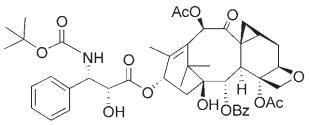
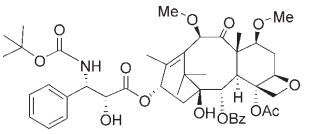
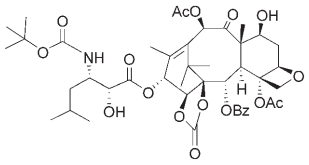
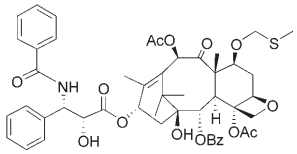
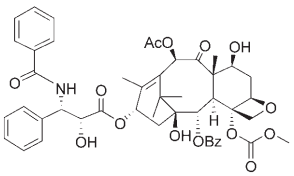
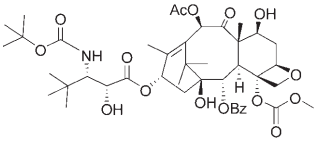
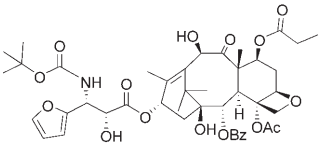
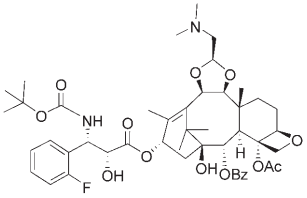
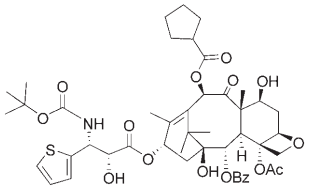
With the aim of identifying compounds more active than paclitaxel against cross-resistant cells, several groups pursued and reported various modifications at the northern hemisphere of paclitaxel, specifically at the C7 and C10 positions, tolerant to chemical manipulations. Wittman and co-workers reported the synthesis of C7 paclitaxel ethers of general structure **35** (Figure 10) which showed cytotoxic activity towards both paclitaxel-sensitive and MDR cell lines, thus demonstrating that their action was not affected by multidrug transporters. Among them, BMS-184476 ($R^1 = R^2 = Ph$, $R^3 = CH_2SCH_3$) was discovered, which exhibited preclinical activity superior to paclitaxel and is now in clinical trials (Table 1).[61]

A huge number of C10 modified paclitaxel analogues were generated by combinatorial chemistry techniques by Georg and co-workers,[62] again with the purpose of reducing the interactions with P-gp, through chemical modifications to the paclitaxel structure. When tested for cytotoxicity and sensitivity to P-gp, taxane **36**, which is characterized by the presence of a succinate group at the C10 position (Figure 10), emerged as the most interesting compound: it retained cytotoxic effects against the breast cancer cell line MCF7 comparable to paclitaxel, and through the lack of enhanced uptake of rhodamine 123, a P-gp substrate, was demonstrated to have no apparent interactions with P-gp. Consequently and as confirmation of the reduced interaction with P-gp, **36** was proved to have an enhanced permeation across the blood-brain barrier.[63]

Tarrant and co-workers described the synthesis of the novel analogues **37 a**–**d** (Figure 11), derived by linking the C7 and C10 positions of the taxane skeleton in a macrocyclic array.[64] Such derivatives were significantly more potent than paclitaxel against the human colon carcinoma cell line HCT116/MDR (which is about 100-fold resistant to the parent drug and is known to express elevated levels of P-gp), thus indicating that they were less susceptible to efflux by P-gp. Compounds **37 a** and **37 c**, when tested in vivo against the ip/ip M109 Madison murine lung carcinoma screen,[64] were shown to possess an activity equivalent to that observed for paclitaxel, whereas compound **37 d**, which displayed reduced in vitro potency, was inactive in the in vivo screen. As a whole, these results suggested that the presence of an unsubstituted macrocycle, while crucial to impair the P-gp-mediated efflux, could be well tolerated at the tubulin binding site.

Concerning macrocyclic derivatives, a number of different taxanes, obtained by connecting different positions of the taxane scaffold in the southern part of the molecule, have been synthesized with the idea that they would provide hybrid constructs of taxanes and epothilones, on the basis of a plausible common pharmacophore for microtubule-stabilizing agents, proposed by Ojima and co-workers.[65] The first series of this type of macrocycles, referred to as C-linked, was synthesized connecting the C2 and C3′ positions of the taxane skeleton (general structure **38** in Figure 11),[66] followed by a further development in this field with the N-linked series of macrocy-

Table 1. Taxanes able to overcome paclitaxel and docetaxel resistance which entered clinical trials.

Index	Name	Structure	MDR[a]	Tubulin alterations[b]	Ref.	Company
61	XRP9881 (RPR-109881A)		yes	not reported	[85]	Aventis Pharma
62	XRP6258 (RPR-116258A)		yes	not reported	[85]	Aventis Pharma
63	IDN5109 (BAY 59-8862)		yes	not reported	[88]	Bayer/Indena
64	BMS-184476		yes	yes	[94]	Bristol-Myers Squibb
65	BMS-188797		no	yes	[94]	Bristol-Myers Squibb
66	BMS-275183		yes	yes	[93]	Bristol-Myers Squibb
67	MAC-321		yes	yes	[95]	Wyeth
68	DJ-927		yes	not reported	[96]	Daiichi Pharmaceuticals
69	MST-997		yes	yes	[97]	Wyeth

[a] Described capability to fully or partially overcome transport-based resistance. [b] Described capability to fully or partially overcome resistance due to tubulin alterations.

clic taxanes (general structure **39** in Figure 11).[67] However, the biological activity of these molecules against P-gp-based MDR expressing cells has not been reported to date, as is the case for the C4 and C3′ constrained macrocyclic paclitaxel analogues prepared by Kingston and co-workers.[68]

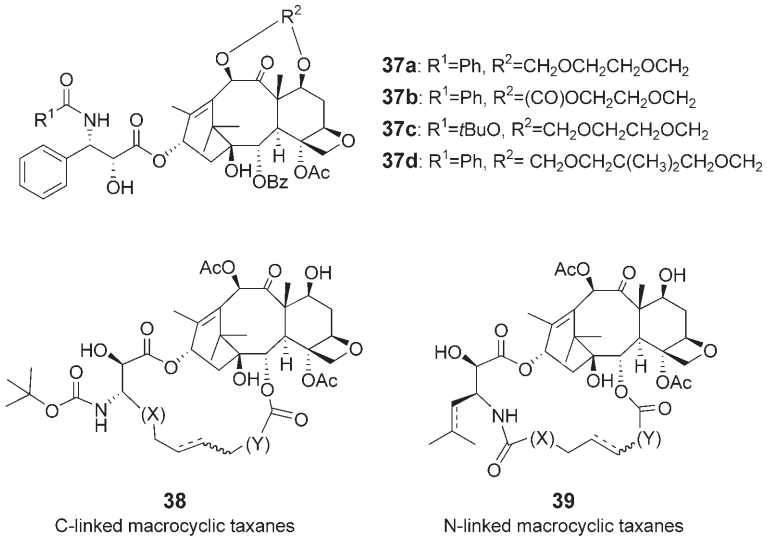


Figure 11. Macrocyclic paclitaxel analogues: only the C7-C10 linked (northern hemisphere) macrocycles are reported to be able to evade P-gp mediated efflux from cells.

In the course of SAR investigations on paclitaxel and docetaxel analogues, various 3′-alkenyl and 3′-alkyl derivatives showed substantially better cytotoxicity than the parent compounds, especially against drug-resistant cells.[69] Further modifications at the C10 position of 3′-(2-methyl-1-propenyl-) and 3′-(2-methylpropyl-)taxanes induced a significant increase in their cytotoxicity against a number of normal cancer cells and drug-resistant MCF7-R cancer cells.[70] The three compounds **40**, **41**, and **42** (Figure 12), in particular, were found to possess excellent potency against the MCF7-R cell line, two orders of magnitude better than those of paclitaxel and docetaxel. A comprehensive analysis of data for this series of derivatives revealed that, although there was a limitation in the optimal size of the C10 acyl group, a variety of acyl substituents seemed to be tolerated in terms of cytotoxicity against normal cancer cells; on the contrary, the activity against the drug-resistant cancer cells strongly depended on the structure of the C10 modifier, suggesting that the C10 position is crucial for P-gp to recognize and bind taxanes.

Ferlini and co-workers reported a study concerning the growth inhibition effect of three second generation taxanes, namely **42**, **43**, and **44** (Figure 12), on a panel of seven human cancer cell lines, in comparison with paclitaxel and docetaxel.[71] Results unambiguously highlighted the exceptional activity of the novel taxanes towards P-gp positive cells (up to >400 fold higher potency than that of paclitaxel), with **42** and **43** shown to be substantially more active than paclitaxel and docetaxel against P-gp negative cells. Remarkably, the rhodamine 123 assay revealed that these taxanes also had the capability to inhibit the function of the P-gp efflux pump, thus demonstrating that new generation taxanes simultaneously possessing cytotoxic and MDR reversal activities represent a feasible approach.

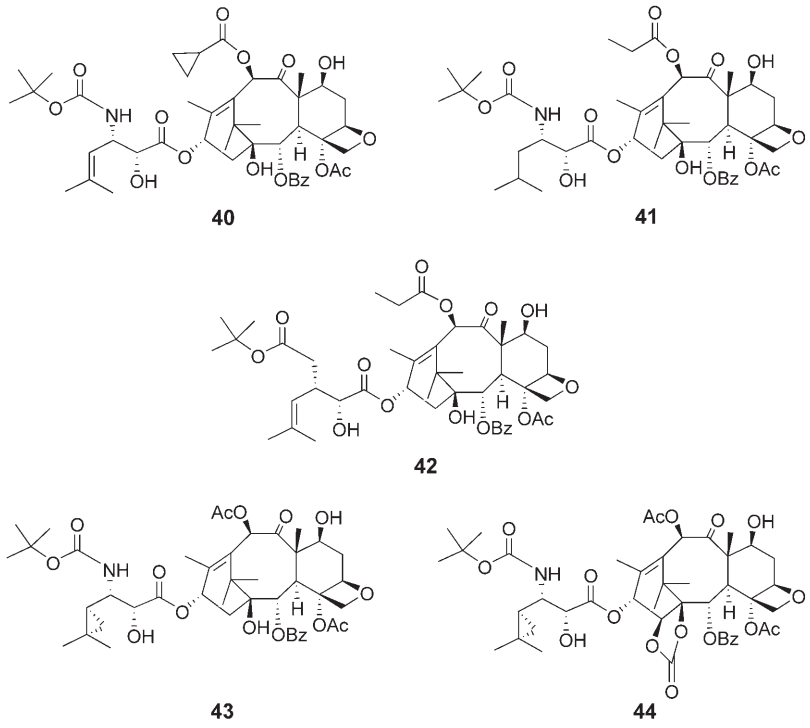


Figure 12. Taxanes bearing modifications at the key positions C10 and C3′ endowed with cytotoxic activity against MDR cell lines. Compounds **42**, **43** and **44** were also reported to modulate the P-gp multidrug transporter.

Among the second generation taxanes, **40**, **42**, and a number of their congeners modified at the C10 and C3′ positions were selected for conjugation with polyunsaturated fatty acids (PUFA).[72] In more detail, the C2′ hydroxy group of these taxanes was coupled to linolenic acid (LNA), linoleic acid (LA), and docosahexaenoic acid (DHA), and the resulting PUFA-taxane conjugates were assayed in vivo against both drug-sensitive human ovarian tumor (A121) and highly drug-resistant colon cancer (DLD-1, overexpressing P-gp) xenografts. Whereas DHA–paclitaxel was shown to be ineffective against

the drug-resistant P-gp overexpressing xenografts, the new conjugates DHA–**40** and LNA–**42**, exhibited strong efficacy against DLD-1 xenografts, and the total regression of drug-resistant and drug-sensitive tumors was achieved with DHA–**40** and DHA–**42**, respectively (Figure 13).

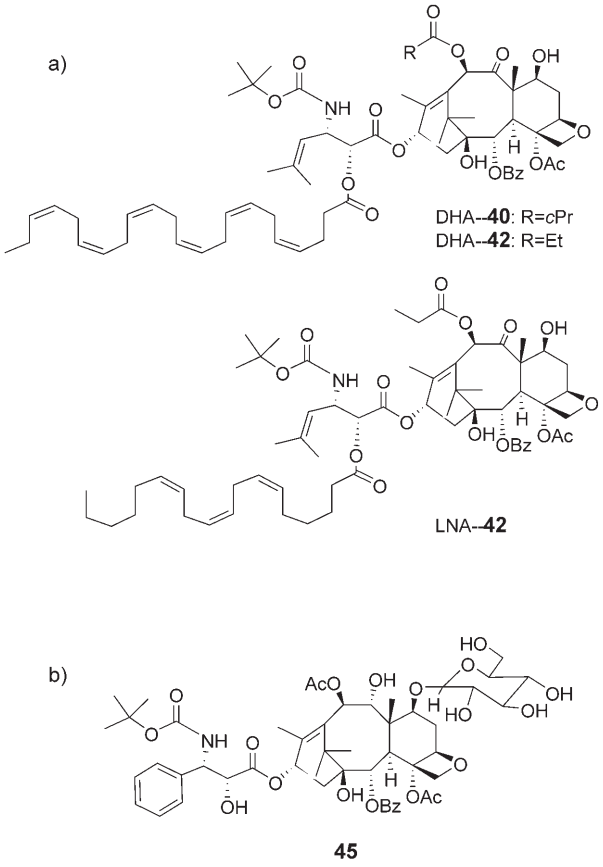


Figure 13. a) PUFA-taxane conjugates; b) example of O-glycosilated docetaxel analogue.

The semisynthesis of a 7β-O-glycosilated docetaxel analogue **45** (Figure 13) has recently been reported by Zamir and coworkers, with the aim of preparing taxane glycoconjugates similar to those found in nature.[73] Compound **45** was found to possess reduced cytotoxicity in the MCF7 cell line compared to paclitaxel, but it demonstrated better activity against the drug-resistant cell line MCF7-ADR.

In the course of studies aimed at improving the activity profile of paclitaxel and docetaxel, different series of seco-taxanes have been synthesized, in which the A and the C rings were separately cleaved, yet retaining the other rings. The in vitro cytotoxicities of the A-seco-taxanes **46**, **47**, and **48** (Figure 14) were evaluated against several human tumor cells, including the MCF7-R resistant line,[74] with compounds **46** and **48** retaining a certain level of cytotoxicity, though exhibiting weaker activity than paclitaxel. Concerning the C-seco paclitaxel analogues, despite the opening of the convex taxane core and a decreased number of stereogenic carbons which make these compounds structurally simpler than most of their chemical analogues, derivatives **49c** and **49d** (Figure 14) were shown to retain a moderate anticancer activity when tested towards both normal (MDA-MB231) and adriamycin-resistant (MCF7-ADR) breast tumor cells.[75] Among them **49c** (IDN5390) was selected for preclinical development, on the basis of its antitumor efficacy on a large panel of human tumor xenografts and oral bioavailability.[76] IDN5390 has also recently been reported to circumvent paclitaxel resistance in drug-resistant cells overexpressing class III β-tubulin (see section 2.2).

The acylative modification of IDN 5390, which resulted in compounds **50a–d** and **51**, was also investigated (Figure 14), though none of the newly synthesized compounds exhibited an increase in potency.[77]

Among the wide range of structural modifications possible on the paclitaxel skeleton, the introduction of small substituents on the 2-benzoate moiety of new taxanes has been reported to increase their cytotoxicity.[78] Consistent with this finding, a series of second-generation taxanes with modifications at the C3′ and C10 positions of paclitaxel and bearing a meta substituent on the 2-benzoate were designed and synthesized: some compounds were found to possess higher potency against drug-resistant cancer cell lines LCC6-MDR and MCF7-R compared with paclitaxel and docetaxel. In particular, three of these taxanes (**52**, **53**, and **54**, Figure 15) showed essentially no difference in activity against drug-resistant and drug-sensitive cell lines, and have been categorized as advanced second-generation taxane anticancer agents.[79]

It has been proposed that this *meta*-effect, that is, the increase in antitumor activity associated with the presence of a substituent at the *meta*-position of 2-benzoate, could be due to a more favorable interaction between the C2 benzoate ring and the Asp 224 residue of tubulin, which constitutes part of the taxane binding pocket.[80,81] The *meta*-effect has been also investigated in the newer generation taxanes IDN5109 (Table 1) and IDN5390 (Figure 14),[82] by measurement of the cytotoxicity of derivatives **55** and **56** (Figure 15) on MCF7 and MCF7-R cell lines. As expected, the *meta*-methoxy group showed a general boosting effect on cytotoxicity in both cell lines, demonstrating that the *meta*-effect has broad generality in anticancer taxanes, even in C-seco analogues that substantially differ from taxanes in terms of topology and conformational properties.

Further modifications at the taxane skeleton have been accomplished on the basis of the previously mentioned common pharmacophore for microtubule-stabilizing agents.[65] This pharmacophore suggested that the baccatin III core (**57**, Figure 16) may serve as a rigid scaffold, able to secure the proper orientation of the C2 and C3′ moieties. Consistent with this idea, the same group described an interesting approach to the design of baccatin-free taxane-mimics, in which the baccatin core was replaced by a much simpler scaffold retaining most of its three-dimensional features, but without its structural complexity.[83] The authors searched several chemical structure databases for bicyclic structures bearing two hydroxy groups that mimic the distance and proper dihedral angle of the C2 and C13 hydroxy groups of baccatin III. The search singled out the indolizidinone alkaloid skeleton **58** (Figure 16),

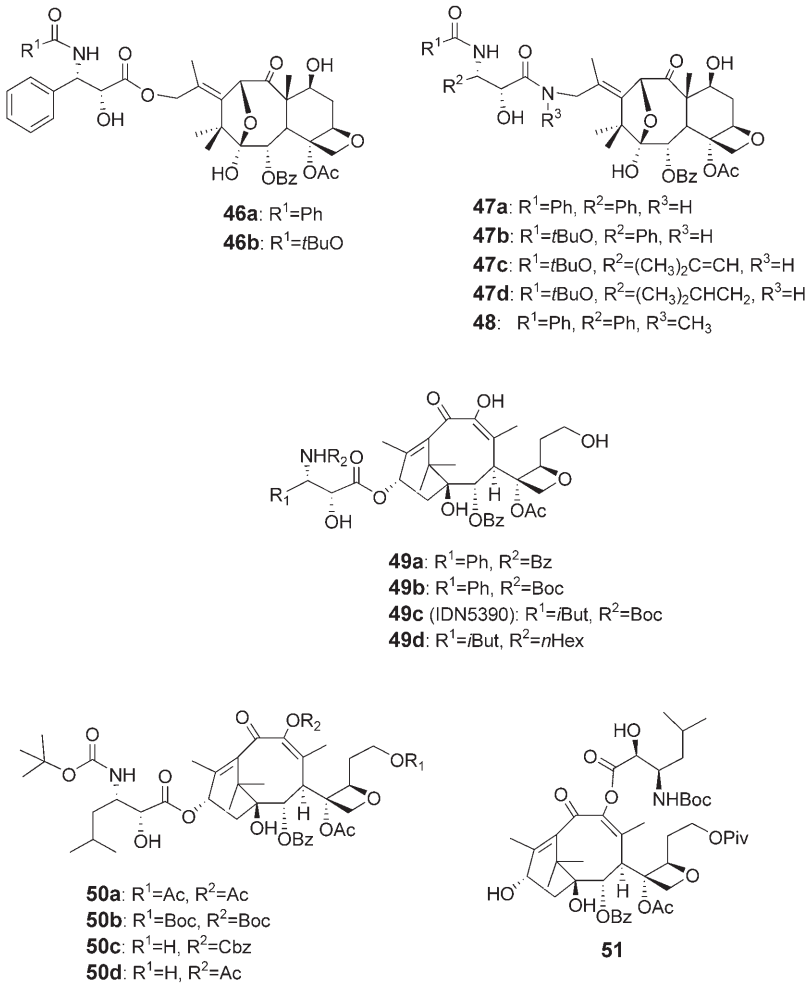


Figure 14. A-seco and C-seco paclitaxel analogues. Only compounds **46a**–**b** and **49c**–**d** exhibited a certain activity against MDR cells.

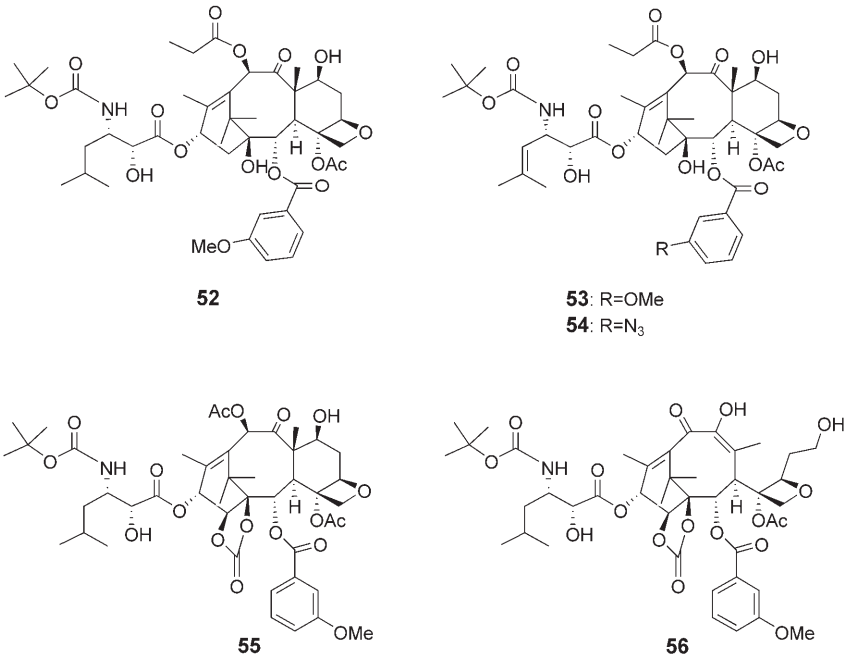


Figure 15. Examples of *meta-effect* in advanced second-generation taxane anticancer agents.

which locates the two crucial hydroxy groups at approximately 5 Å distance and with a dihedral angle of 40 to 50°, a relative orientation that closely resembles the two crucial oxygen atoms at the C2 and C13 positions of the baccatin core as found in the X-ray structure of docetaxel[84] and in the energy-minimized models of paclitaxel and other taxanes. Using the baccatin-mimicking scaffold **58**, new de novo taxanes such as **59** and **60** have been prepared (Figure 16). These compounds appeared not to be affected by P-gp-based cross-resistance and compound **59** exhibited a cytotoxicity comparable to cisplatin; however, none of them were found to appreciably promote the formation of microtubules, and it was suggested that their cytotoxic activity could be due to a different mechanism of action.

It is worth mentioning that a number of synthetic taxanes (**61**–**69**) endowed with improved activity against cross-resistant tumors compared to paclitaxel or docetaxel entered clinical trials (Table 1). It was shown that these agents were either not, or only marginally affected by multidrug transporter-mediated efflux from cells and usually exhibited better pharmacokinetic properties than parental drugs. In addition, some of them also overcame resistance caused by target alteration mechanisms (see Section 2.1).

XRP9881 (RPR-109881A, **61**) and XRP6258 (RPR-116258A, **62**), developed by Sanofi–Aventis, were demonstrated to be active in cell lines resistant to paclitaxel, docetaxel, and other tubulin targeting agents, and to possess a lower affinity for P-gp in comparison to docetaxel, together with the ability to penetrate the blood-brain barrier.[85,86] On the basis of the pattern of cytotoxicity and lack of cross-resistance

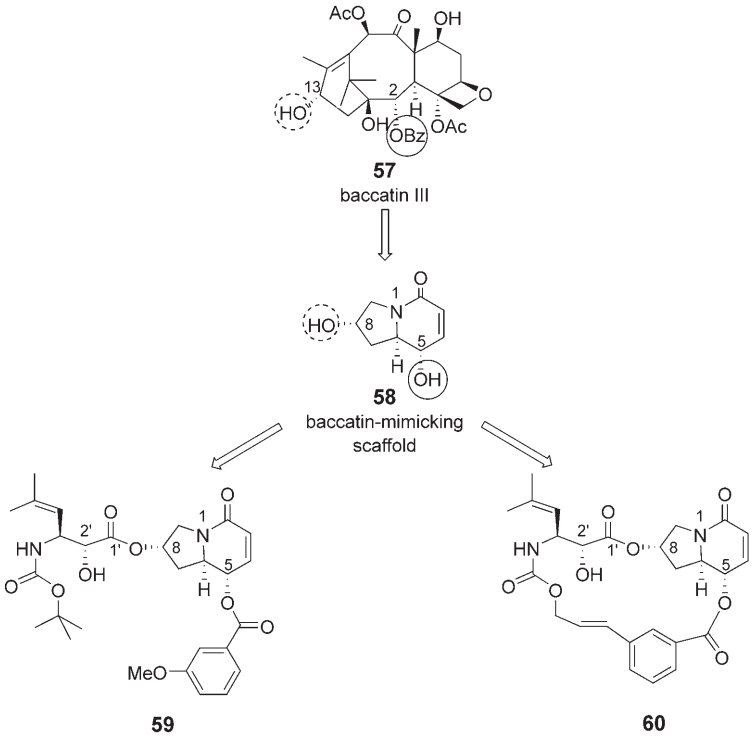


Figure 16. De novo taxanes derived from the indolizidinone scaffold mimicking the baccatin core. Solid and dashed circles label the two functional groups which are superimposed in the 3D space by the different scaffolds.

in tumor cell lines expressing the typical MDR phenotype, the carbonate derivative ortataxel (formerly IDN5109 and BAY 59-8862, **63**)[87] was selected for clinical development among taxanes derived from 14β-OH-DAB. In a comparative efficacy study of IDN5109 and paclitaxel, performed on a large panel of human tumor xenografts, IDN5109 exhibited a superior activity against tumors resistant to paclitaxel and a superior or comparable activity against paclitaxel-responsive tumors.[88] Importantly, IDN-5109 was also demonstrated to modulate the activity of P-gp (and, more recently, also of other MDR-associated ABC transport proteins),[89] and this capability was proposed to account for the superior tumor growth inhibition against cross-resistant tumors compared with paclitaxel.[90] Modification of paclitaxel at C7 and docetaxel at the C4 and C3′ positions led to the discovery of BMS-184476 (**64**)[91] and BMS-275183 (**66**)[92], respectively. In comparison studies, the level of resistance exhibited by the P-gp overexpressing paclitaxel-resistant cell line HCT-116/MDR to both compounds was significantly less severe than the resistance manifested towards the parent drug.[93,94] MAC-321 (**67**), developed by Wyeth Research, is a docetaxel analogue sharing some biochemical properties with paclitaxel and docetaxel. It was shown to overcome drug resistance in tissue culture or animal models, and the weak interaction with P-gp was proposed as one of the reasons for its interesting activity; consistent with this hypothesis, epidermoid KB-8-5 tumor cells expressing low to moderate levels of P-gp retained sensitivity to MAC-321 compared to paclitaxel and docetaxel.[95] The taxane DJ-927 (**68**) has been demonstrated to overcome in vivo and in vitro MDR mediated by P-gp, and to be more effective than paclitaxel and docetaxel in various tumor cell lines. The cytotoxicity of DJ-927 was not influenced either by the P-gp expression levels of cancer cells or by the presence of a P-gp modulator and it was also shown to be better accumulated in cells and less efficiently exported by P-gp compared to paclitaxel and docetaxel, leading to the assumption that this agent should not be a substrate for this transporter.[96] MST-997 (**69**) is a novel taxane, recently reported as a tubulin polymerizing agent more potent than paclitaxel and able to overcome paclitaxel resistance in epidermoid and colorectal cancer cell lines overexpressing the P-gp efflux pump. MST-997 also showed superior efficacy to paclitaxel and docetaxel on cell lines harboring point mutations in tubulin (see section 2.1) and on paclitaxel- and docetaxel-resistant xenografts.[97]

2. Taxane resistance associated with alterations of the tubulin/microtubule system

Besides transport-based MDR mechanisms, in recent years resistance to taxanes (especially to paclitaxel) has also been frequently associated with alterations of their cellular target, namely the tubulin/microtubule system.[3,98]

Microtubules are filamentous polymers of cytoskeleton, consisting of α/β-tubulin heterodimers; these heterodimers assemble to form linear protofilaments and about 13 protofilaments associate in parallel to form hollow tubes.[99] Microtubules are involved in several cellular functions, including motility, morphogenesis, intracellular transport, mitosis, and meiosis. An essential feature for the activity of microtubules is their so-called dynamic instability; microtubules are in fact highly dynamic structures, being dimers continuously incorporated into the microtubule (polymerization) and released in solution in the cells (depolymerization).[100,101] Paclitaxel binds to the β-tubulin subunit in polymerized microtubules, resulting in suppression of microtubule dynamics and stabilization of the microtubules themselves, with differential effects depending upon the binding stoichiometry.[102,103]

Alterations of the tubulin/microtubule system that have been proposed to confer taxane resistance include tubulin mutations, changes in the expression levels of either β-tubulin isotypes or microtubule regulatory proteins such as microtubule associated proteins (MAPs) or stathmin, post-translational modifications involving tubulin subunits or regulatory proteins, reduction of tubulin content in cells. In most cases, such alterations are supposed to induce resistance either by affecting the drug binding site, thus diminishing the efficacy of binding, or by increasing the dynamicity and reducing the stability of microtubules, thus counteracting the stabilizing effects of paclitaxel.

It is worth noting that attention has only been focused on this kind of resistance more recently, in comparison with resistance based on transport mechanisms; as a consequence, the

number of taxanes reported as able to overcome resistance associated with tubulin/microtubule system alterations is still quite restricted. In addition, the role of these distinct mechanisms of resistance, in particular their contribution to resistance in clinical oncology, is still to be fully understood.

2.1. Point mutations in tubulin

Several point mutations of tubulin have been associated with paclitaxel resistance (Table 2). These mutations can directly affect the binding of paclitaxel or alternatively they can alter longitudinal/lateral interactions between tubulin heterodimers and the binding of regulatory proteins, resulting in more dynamic microtubules. As the paclitaxel binding site is located in the β-tubulin subunit, the search for tubulin mutations has mainly been restricted to the β-tubulin sequence. In fact, most of the documented mutations concern the major β-tubulin isotype, namely class I β-tubulin;[104–107] however, it has been shown that mutations in paclitaxel-resistant cell lines may occur not only in β- but also in α-tubulin.[108–110] Remarkably, despite the fact that numerous in vitro studies involving different cancer cell lines have shown correlations between the presence of tubulin mutations and resistance to paclitaxel and to other tubulin targeting agents (such as epothilones or vinca alkaloids), the significance of tubulin mutations in clinical drug resistance remains unclear.[111]

The first tubulin point mutations conferring resistance to paclitaxel in human cells were found in two paclitaxel-resistant sublines (1A9PTX10 and 1A9PTX22) derived from the 1A9 human ovarian carcinoma cells.[104] These cell lines did not express P-gp and the dynamics of their microtubules was not altered. Sequence analysis revealed two different point mutations in class I β-tubulin: $\beta270^{Phe\rightarrow Val}$ in clone 1A9PTX10 and $\beta364^{Ala\rightarrow Thr}$ in clone 1A9PTX22. The structure of tubulin in complex with paclitaxel,[81] obtained by electron crystallography (EC) and reported in Figure 17, indicates that both such mutations are likely to alter the drug binding, thus preventing paclitaxel-induced tubulin polymerization: in fact, β270 is one of the residues constituting the taxane binding pocket, making direct contact with the ligand; likewise β364 (alanine in human βI-sequence, serine in wild-type pig brain tubulin used for the EC structure determination), although not in direct contact with paclitaxel, is part of a hydrophobic cluster (also including Phe 270) whose rearrangement would result in a less efficacious interaction of the ligand with its target.[112]

A cluster of mutations affecting leucines 215, 217, and 228 were detected by an analysis of class I β-tubulin mutations in Chinese hamster ovary (CHO) cells resistant to paclitaxel.[105] As shown in Figure 17, such residues are located in a region of β-tubulin (β215 and β217 are in loop H6–H7, β228 falls within the H7 helix) which is supposed to play a crucial role not only for the ligand binding, but also for the modulation of microtubule dynamics, being involved in tubulin subunit–subunit interactions, which are important for microtubule stability, and in the switch from the straight (stable microtubules) to the curved (depolymerizing microtubules) conformation of tubulin.[113–115] A number of observations led to the conclusion that mutations of leucine residues that induced paclitaxel resistance are likely to destabilize microtubules by weakening interactions between tubulin subunits during microtubule assembly and/or by preventing putative conformational changes in tubulin due to paclitaxel binding. A further study from the same group investigated the effects of different mutations affecting leucine 215 and reported the first mammalian mutation ($\beta215^{Leu\rightarrow Ile}$) able to enhance sensitivity to paclitaxel.[106] The same study also confirmed the relevance of the H6–H7 loop for microtubule assembly and thereby for the mechanism of action of paclitaxel and for the development of resistance to paclitaxel itself.

In a recent study,[107] a novel point mutation ($\beta26^{Asp\rightarrow Glu}$) was identified in class I β-tubulin of the paclitaxel-resistant cell line KB-15-PTX/099, which derived from the epidermoid carcinoma cell line KB-3-1, did not express P-gp and exhibited impaired microtubule stability compared with parental cells. β26 is part of the drug-binding site[81] and can favorably interact with groups at C2′ and C3′ on the C13 side chain of paclitaxel (Figure 17); replacement of aspartate with glutamate would result in the

Table 2. Tubulin mutations associated with resistance to paclitaxel.

Mutation	Cell line	Agent used for selection	Ref.
$\alpha195^{Leu\rightarrow Met}$	A549.EpoB480	epothilone B	[110]
$\alpha379^{Ser\rightarrow Arg}$	A549-T12	paclitaxel	[109]
	A549-T24	paclitaxel	[109]
$\beta26^{Asp\rightarrow Glu}$	KB-15-PTX/099	paclitaxel	[107]
$\beta60^{Val\rightarrow Phe}$	A549.EpoB480	epothilone B	[110]
$\beta173^{Pro\rightarrow Ala}$	HeLa.EpoA9	epothilone A	[118]
$\beta215^{Leu\rightarrow His}$	paclitaxel-resistant CHO mutants	paclitaxel	[105]
$\beta215^{Leu\rightarrow Arg}$			[105]
$\beta215^{Leu\rightarrow Phe}$			[105]
$\beta215^{Leu\rightarrow Ala}$			[106]
$\beta215^{Leu\rightarrow Glu}$			[106]
$\beta215^{Leu\rightarrow Met}$			[106]
$\beta217^{Leu\rightarrow Arg}$			[105]
$\beta228^{Leu\rightarrow Phe}$			[105]
$\beta228^{Leu\rightarrow His}$			[105]
$\beta231^{Ala\rightarrow Thr}$	CEM/dEpoB	desoxyepothilone B	[119]
$\beta270^{Phe\rightarrow Val}$	1A9PTX10	paclitaxel	[104]
$\beta274^{Thr\rightarrow Ile}$	1A9/A8	epothilone A	[117]
$\beta282^{Arg\rightarrow Gln}$	1A9/B10	epothilone B	[117]
$\beta292^{Gln\rightarrow Glu}$	A549.EpoB40	epothilone B	[118]
	A549.EpoB480	epothilone B	[110]
$\beta292^{Gln\rightarrow Glu}$	CEM/dEpoB	desoxyepothilone B	[119]
$\beta364^{Ala\rightarrow Thr}$	1A9PTX22	paclitaxel	[104]
$\beta422^{Tyr\rightarrow Cys}$	HeLa.EpoB1.8	epothilone B	[118]

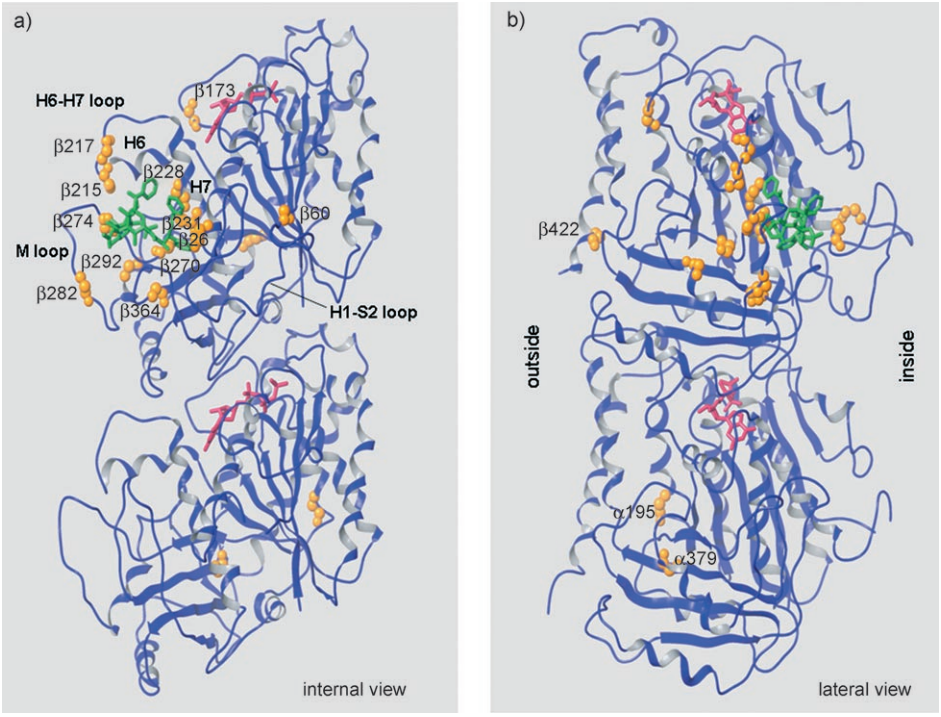


Figure 17. Ribbon representation of the α/β-tubulin heterodimer (derived from 1JFF of the Brookhaven Protein Data Bank),[81] with α-tubulin at the bottom and β-tubulin at the top: a) Internal view, from the inside of the microtubule; b) lateral view, as seen from the left side of Figure a. Backbone atoms of residues which have been reported to undergo mutations in taxane-resistant cells are represented as orange spheres; paclitaxel is represented as green sticks, GTP (in α-tubulin) and GDP (in β-tubulin) are represented as magenta sticks.

partial loss of such interactions, together with a displacement of the ligand somewhat out of the binding pocket, suggesting that resistance could arise from a less efficacious binding of paclitaxel to tubulin. On the other hand, the same mutation was associated with less stable microtubules, indicating that the alteration of microtubule dynamics could play a relevant role in the decreased sensitivity of this cell line to paclitaxel. Taken together, these findings made $\beta26^{Asp\rightarrow Glu}$ the first reported mutation involving both the impaired efficacy of drug binding and the altered microtubule stability in paclitaxel resistance. In agreement with the importance of Asp 26 for paclitaxel binding and tubulin function, the $\beta26^{Asp\rightarrow Glu}$ mutation was also associated with paclitaxel resistance in CHO cells.[116]

A point mutation affecting the α-tubulin subunit was discovered in two human non-small cell lung carcinoma (NSCLC) cell lines resistant to paclitaxel, namely A549-T12 and A549-T24.[109] Such cells, whose microtubules displayed increased dynamicity in the absence of paclitaxel, were characterized by the presence of the $\alpha379^{Ser\rightarrow Arg}$ mutation in the major α-tubulin isotype, Kα1, whereas sequencing of the class I β-tubulin did not reveal any mutation. Both wild type and mutant tubulin were expressed in A549-T12 and A549-T24 cell lines. In the α-tubulin structure, α379 is situated near the C terminus, on the outside of the microtubule (Figure 17), in a region which has been proposed to interact with tubulin regulatory proteins MAP4 and stathmin, microtubule stabilizer and destabilizer, respectively. Therefore, mutation at α379 may affect the binding of these regulatory proteins to tubulin, thus affecting the dynamics of microtubules and inhibiting the stabilizing properties of paclitaxel. Moreover, altered expressions of stathmin and MAP4 proteins were detected in the same cells, as described below; these changes, which may cause additional destabilization of the microtubule network, were hypothesized to be related to the α-tubulin mutation and to contribute to the paclitaxel-resistant phenotype.

Additional point mutations have been described in studies on different human carcinoma cell lines that were selected for resistance to epothilones, but also exhibited cross-resistance towards taxanes. These mutations are: $\beta274^{Thr\rightarrow Ile}$ and $\beta282^{Arg\rightarrow Gln}$ in the ovarian cell lines 1A9/A8 and 1A9/B10, respectively;[117] $\beta292^{Gln\rightarrow Glu}$, $\beta173^{Pro\rightarrow Ala}$, and $\beta422^{Tyr\rightarrow Cys}$ in the non-small cell lung A549.EpoB40 and in the cervical HeLa.EpoA9 and HeLa.EpoB1.8 cancer cells, respectively;[118] $\beta231^{Ala\rightarrow Thr}$ and $\beta292^{Gln\rightarrow Glu}$ in the leukemia cells CEM/dEpoB;[119] $\beta292^{Gln\rightarrow Glu}$, $\beta60^{Val\rightarrow Phe}$, and $\alpha195^{Leu\rightarrow Met}$ in the non-small cell lung line A549.EpoB480.[110] Epothilones are natural products whose structures are unrelated to those of taxanes; notwithstanding that they share with taxanes a common mechanism of action and a common binding pocket in β-tubulin.[120,121] All the residues implicated in the mutations mentioned above are displayed in Figure 17. β274 and β282 belong to the M loop, which constitutes part of the taxane/epothilone binding pocket and is also fundamental for lateral interactions between tubulin subunits.[117] Likewise β292 is near both the M loop and Thr 274, a key amino acid residue in the binding pocket; β231 is located in the H7 helix, within the ligand-binding site but also in a region involved in regulating the conformation of tubulin end thereby the microtubule stability. β173 is on a loop which forms part of the nucleotide binding site; as described elsewhere, binding and hydrolysis of GTP occurring at this site constitute an essential regulatory mechanism of microtubule dynamics.[99] β422 and α195 are both located at the external surface of the microtubule, which is thought to be the domain that interacts with microtubule regulatory proteins. Finally, β60 sits at the end of the H1–S2 loop, which interacts with the M loop of an adjacent tubulin molecule and like the M loop has a substantial role in modulating lateral contacts between tubulin subunits. Similar to the mutations previously described, those also conferring resistance to both epothilones and taxanes are located in sites of tubulins which are involved either in the binding of ligands ($\beta274^{Thr\rightarrow Ile}$) or in the regulation of microtu-

bule stability (β173$^{Pro\rightarrow Ala}$, β422$^{Tyr\rightarrow Cys}$, α195$^{Leu\rightarrow Met}$, and β60$^{Val\rightarrow Phe}$), or in both (β282$^{Arg\rightarrow Gln}$, β292$^{Gln\rightarrow Glu}$, and β231$^{Ala\rightarrow Thr}$).

The number of reported taxanes which maintain their efficacy in paclitaxel or docetaxel-resistant cell lines harboring tubulin mutations is quite restricted. Among a set of 2-aroyl analogues of paclitaxel synthesized to obtain derivatives endowed with enhanced activity, compounds **70a**–**d** (Figure 18) exhibited the most interesting behavior, being able to partially overcome paclitaxel resistance in the ovarian carcinoma cell line 1A9PTX22, although none of them could completely reverse it.[122]

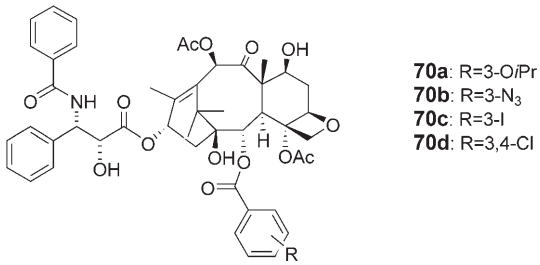


Figure 18. 2-aroyl paclitaxel analogues able to partially overcome taxane resistance due to altered β-tubulin.

A number of taxanes that entered clinical trials displayed less cross-resistance in tubulin-mutant cell lines in comparison with paclitaxel, usually associated with an increased activity against cells overexpressing drug efflux pumps (for this reason, most of them have already been described before) and with improved pharmacokinetic properties (Table 1). In this context, substantially reduced, but still significant resistance to clinical candidates BMS-184476,[93] BMS-188797,[93] and BMS-275183[94] was found in the paclitaxel-resistant cells A2780/tax,[104] harboring point mutations in β-tubulin. Lower levels of cross-resistance relative to parental cells (1.6- to 10.7-fold) were observed for taxane MAC-321 in tumor lines 1A9PTX10, 1A9PTX22, 1A9/A8, and A549.EpoB40, when compared with paclitaxel (4.1- to 20.8-fold), whereas comparable or lower levels were observed when compared with docetaxel (1.2- to 16.6-fold);[95] similar results were found for MAC-321, paclitaxel, and docetaxel also in the KB-15-PTX/099 cell line, which exhibited 3.9-, 18.2-, and 4.9-fold resistance to these agents, respectively.[107] Likewise, the novel taxane MST-997 was demonstrated to be more active than paclitaxel and docetaxel in A549.EpoB40 and KB-15-PTX/099 cell lines, displaying significantly reduced cross-resistance.[97] It has been hypothesized that such agents may interact with tubulin in a binding site similar but distinct from that of the epothilones and most taxanes,[95,97] or that they could interact more efficaciously than paclitaxel with the mutant tubulin;[107] however, the exact molecular mechanism by which these taxane derivatives could overcome resistance induced by tubulin mutations is still to be fully elucidated.

As anticipated, although β-tubulin mutations have been shown to play a role in resistance in several cultured cell lines exhibiting resistance to paclitaxel and other tubulin stabilizing agents, to date there is little evidence that they can be important for the development of clinical resistance.[111]

The first β-tubulin mutations in a clinical setting were described in a study on tumor samples from NSCLC patients;[123] however, following studies indicating that the results of this report (that suggested a relationship between the presence of β-tubulin mutations and a lack of response to paclitaxel-based chemotherapy) should be handled with caution, as the PCR primers used for the study were also demonstrated to be capable of amplifying β-tubulin pseudogenes, leading to the interpretation of such nonfunctional genes as mutations of the wildtype sequences.[124,125] Furthermore, a number of studies on various patient sets did not corroborate the findings previously described and concluded that β-tubulin mutations in clinical samples are either absent or are so rare as to be unlikely to contribute to paclitaxel resistance.[124–129] Nevertheless, data obtained from cell lines can not be ignored, and suggest that further investigation of clinical samples is required before definitively excluding that tubulin mutations can constitute a clinically relevant mechanism of resistance to taxanes.

2.2. Changes in the expression levels of β-tubulin isotypes

In the last few years, modifications of the tubulin/microtubule target by differential expression of β-tubulin isoforms have been proposed by numerous reports as a mechanism of resistance to taxanes and other antimitotic drugs.[3,98,130] In humans, seven distinct β-tubulin isotypes (classes I, II, III, IVa, IVb, V, and VI) have been detected;[131,132] they constitute a highly homologous protein family and most of the differences among their sequences reside in the C-terminal amino acids, which are exposed at the surface of microtubules and undergo post-translational modifications. The expression profile of the isotypes is different among tissues, but we are still far from a full comprehension of their exact functional significance. It has been shown in vitro that the tubulin isoform composition can influence the dynamic properties of microtubules as well as their sensitivity to paclitaxel; in particular, microtubules assembled from purified α/βIII-tubulin were considerably more dynamic than those made from unfractionated tubulin or from the purified α/βII- or α/βIV-tubulin[133] and microtubules composed of purified α/βIII- and α/βIV-tubulin were less sensitive to the action of paclitaxel than those assembled from α/βII- or unfractionated tubulin.[134]

There are several studies on both cell lines and clinical samples in which the altered expression levels of β-tubulin isoforms (detected either at the mRNA level or at the protein level or both), especially the increased expression of class III β-tubulin, was associated with resistance to paclitaxel and also to docetaxel, strongly suggesting that this kind of alteration can give rise to the resistance phenotype.

Overexpression of class III and IVa β-tubulin was found in two paclitaxel-resistant human lung carcinoma cell lines (A549-T12 and A549-T24 NSCLC cell lines), which correlated with a reduced sensitivity to paclitaxel.[135] The same study also revealed similar changes in tubulin expression levels in clinically derived ovarian epithelial tumor cells resistant to paclitaxel, and was

the first report indicating that an increased expression of specific β-tubulin isoforms can induce clinical resistance to the drug. A twofold increase in class IVa β-tubulin was detected in a erythroleukemia cell line (KPTA5) resistant to paclitaxel,[136] overexpression of class V was proposed to confer paclitaxel resistance in CHO cells,[137] and altered expression of various isotypes was reported for breast cancer cells resistant to docetaxel.[138] Besides lung carcinoma,[135] enhanced expression of class III β-tubulin isotype in cell lines was observed even in other tumor types endowed with a taxane-resistant phenotype, including prostate, breast, and ovarian carcinoma cells;[139–141] it was associated with resistance to paclitaxel also in a subset of 17 human cell lines from the National Cancer Institute Anticancer Drug Screen and in CHO cells.[142,143] The hypothesis that the βIII-tubulin isotype may be involved in resistance towards taxanes was further supported by a study on human brain carcinoma cell lines (SNB75, SF295, and SF539) with different intrinsic levels of βIII isoforms and not selected for drug resistance; it was shown that cells with elevated levels of class III β-tubulin were significantly less sensitive to paclitaxel compared to the cells with no detectable levels of such isotype.[144]

A few studies focused on the role of α-tubulin isotypes in taxane resistance (similar to β isotypes, six distinct α-tubulin isotypes have been reported and their functional role is not well known);[131] however, overexpression of α-tubulin was described in the lung carcinoma cell line H460/T800, which was 1000-fold more resistant to paclitaxel compared to the parental cell line and also overexpressed P-gp.[145]

Unlike tubulin mutations, a meaningful number of studies have described altered β-tubulin isotype expression levels, mainly involving the βIII isoform, in different clinical samples, and correlated them with acquired taxane resistance; nevertheless, the data currently available does not allow an exhaustive comprehension of the relevance of this type of target alteration in clinical resistance to taxane chemotherapy, and also the molecular mechanism by which the modified isotype composition of microtubules could mediate resistance has still to be elucidated.

As anticipated before, an analysis of β-tubulin isoforms in patients with ovarian cancer revealed that paclitaxel-resistant tumor samples displayed significant increases in class I, III, and IVa β-tubulin isotypes compared with untreated tumors.[135] On the other hand, no correlation was observed between β-tubulin isoform expression and paclitaxel sensitivity in 12 human ovarian carcinoma xenografts established from samples taken before or after paclitaxel treatment.[142] In a more recent study, the overexpression of class III β-tubulin was associated with the paclitaxel-resistant phenotype in ovarian cancer patients, and was indicated as a prominent mechanism of resistance to paclitaxel in ovarian cancer.[146] However, the same group recently reported the lack of association between βIII-tubulin levels and response to platinum/paclitaxel chemotherapy in ovarian cancer patients, even if the clinical setting was completely different.[147] In a preliminary study on patients with breast cancer, increased expression levels of class I and III β-tubulin were related to docetaxel resistance, and suggested as useful predictors of response to the drug.[148] These findings were confirmed by a recent report from the same group, which showed that class III isotype expression in human breast cancers was significantly associated with resistance to docetaxel.[149] Similarly, still in breast cancers, overexpression of βIII isotype was correlated with resistance to taxanes in patients treated with paclitaxel-containing regimens.[150] Class III β-tubulin has recently been proposed to play a relevant role in determining the clinical resistance to docetaxel- and paclitaxel-based therapy in gastric cancer[151] and in carcinomas of unknown primary site,[152] respectively, and its overexpression has been associated with resistance towards taxanes and with poor prognosis also in NSCLC patients receiving a taxane-based chemotherapy.[153,154]

In this context, the seco-taxane IDN5390 has recently been reported to be more active than paclitaxel in paclitaxel-resistant cell lines overexpressing class III β-tubulin and to exhibit a synergistic activity when combined with paclitaxel in these cells.[142] IDN5390 could therefore represent the prototype of a new class of taxanes endowed with selective activity in cells resistant to paclitaxel owing to an increased expression of βIII-tubulin isotype.

The mechanism by which the overexpression of class III β-tubulin could result in taxane resistance has still to be fully elucidated. It was shown that increased βIII expression can impair microtubule stability both in vitro and in cells.[134,143] Incorporation of βIII isotype into microtubules could make them more dynamic either directly, by weakening interactions between heterodimers, or in a less direct manner, by altering the interactions of tubulin with microtubule regulatory proteins. In both cases, the constitutive destabilization of microtubules induced by the presence of βIII-tubulin would counteract the stabilization induced by taxanes and could therefore constitute a mechanism of resistance.[3] Alternatively, if differences in sequence among isoforms are located in the proximity of the taxane binding site, these could result in a lower affinity of class III β-tubulin towards the ligands and in a less efficacious binding. In this context, a recent study on CHO cells showed that increased levels of βIII-tubulin did not affect the dynamicity of microtubules in the absence of paclitaxel, whereas only in the presence of paclitaxel microtubules of cells overexpressing βIII isoform were significantly more dynamic than those of control cells, suggesting that increased expression levels of class III could induce paclitaxel resistance by reducing its ability to suppress microtubule dynamics. The decreased sensitivity to paclitaxel which characterizes βIII-tubulin overexpressing cells is supposed to be due either to a reduced ligand binding or to a reduced capability to induce conformational changes that would result in altered microtubule dynamics. Consistent with this hypothesis, a recent study of our research group based on docking and molecular dynamics techniques suggested that the resistance to paclitaxel and the different activities of paclitaxel and IDN5390 against microtubules expressing variable levels of βIII-tubulin isotype could be explained in terms of different efficacy of binding of ligands, which can in turn be mainly attributed to the difference in residue β275 (277 in the EC structure) between the two isoforms; β275, which is located in the M loop and is part of the taxane binding site,[81] is serine

in βI and alanine in βIII.[155] In detail, the binding energies calculated in the course of molecular dynamic simulations indicated a higher affinity of paclitaxel for the βI isoform than for the βIII, and an opposite behavior for IDN5390. Analysis of structures derived from molecular modeling corroborated these findings, and suggested that in the βIII-tubulin the replacement of serine with alanine at position 275 would result in a rearrangement of the M loop whose final outcome would be a less efficacious interaction with paclitaxel; conversely, the same rearrangement would induce an increased affinity for IDN5390. As a whole, our results provided a possible explanation at the molecular level for the resistance to paclitaxel associated with βIII-tubulin overexpression and for the interesting activity of IDN5390 towards this isotype.

2.3. Altered expression/post-translational modifications of microtubule regulatory proteins

Regulatory proteins constitute a fundamental component of the tubulin/microtubule system. They modulate microtubule dynamics by interacting either with tubulin heterodimers or with polymerized microtubules, and can therefore influence the sensitivity of cells towards taxanes. Two well characterized microtubule assembly regulatory proteins are MAP4 (the predominant human non-neuronal MAP) and stathmin; the former binds to polymerized microtubules stabilizing them, whereas the second sequesters soluble tubulin dimers and destabilizes microtubules.[156] The activity of these two proteins is regulated by phosphorylation,[157,158] both being inactive in the phosphorylated form. The decreased expression of the microtubule-stabilizing (active) form of MAP4 and/or the increased levels of the active form of stathmin should enhance the dynamicity and reduce the stability of the microtubule network. As a consequence, such alterations are likely to hinder the stabilizing action of taxanes and to confer a mechanism of resistance to these agents. In fact, stathmin was showed to inhibit in vitro paclitaxel-induced polymerization of microtubules,[159] and enhanced expression levels of this protein were detected in ovarian cancer cells resistant to paclitaxel.[160] On the other hand, increased expression of MAP4 has been associated with increased sensibility to paclitaxel in murine fibroblasts,[161] whereas its repression was demonstrated to result in decreased sensitivity to the drug in the same cells.[162] Analogously, as anticipated above, the two paclitaxel-resistant human NSCLC cell lines A549-T12 and A549-T24 harboring an α-tubulin mutation exhibited a ~twofold increase of the active unphosphorylated form of stathmin and an enhanced phosphorylation of MAP4.[109] To date, however, although altered expression levels of microtubule regulatory proteins have been shown to be involved in paclitaxel resistance, the restricted number of reports on cell lines together with the absence, to our knowledge, of data from clinical samples does not permit a proper assessment of the impact of this mechanism on resistance towards taxanes.

2.4. Tubulin post-translational modifications

Post-translational modifications (polyglutamylation, polyglycylation, and phosphorylation in both α and β subunits, acetylation, reversible tyrosination, and removal of the penultimate glutamate on α subunits) mainly occur within the C-terminal portion of tubulins and contribute to further increase their structural diversity.[131] These modifications constitute another factor that can affect microtubule dynamics: in fact, it is possible that some of them could modulate the stability of microtubules either directly or by influencing the interactions with regulatory proteins (in this regard, there is an increasing body of evidence that post-translational modifications can regulate the association of MAPs with microtubules).[163] For this reason, such modifications could be involved in mediating resistance towards taxanes as well as other tubulin binding agents. Increased acetylation of α-tubulin has been detected in a paclitaxel-resistant human small-cell lung cancer (SCLC) cell line,[164] even though it does not seem associated with resistance to the drug. However, also in this case the studies focused on post-translational modifications of tubulins in resistant cells are rare, thus the role played by these tubulin alterations in taxane resistance remains unclear.

2.5. Reduction in tubulin content

It is worth mentioning that in a recent study the reduction in tubulin content in the cells has been proposed as a novel potential mechanism of paclitaxel resistance.[165] In this report CHO cells (revertants of Cmd 4, a colcemid-resistant CHO cell line) resistant to paclitaxel have been described, which exhibited one-third less tubulin compared to wild-type cells and normal microtubule assembly. The mechanism by which a reduction in tubulin content may result in a resistant phenotype is unknown, though in this case changes in microtubule dynamics or stability do not seem to be involved.

Conclusions

Cellular resistance to taxanes is a very complex phenomenon involving different molecular mechanisms, such as overexpression of multidrug efflux pumps affecting intracellular drug accumulation, alterations of tubulin inducing a reduced sensitivity to the drugs, alterations in the signaling pathways, in the cell cycle, and in the control of apoptosis. Despite several of such mechanisms having been observed and characterized in laboratory models of cancer, the contribution of the distinct resistance phenotypes as well as their role in clinical oncology have yet to be fully evaluated. In this regard, as our knowledge on mechanisms of resistance increases, it becomes evident that the assessment of their roles in human tumors is a very challenging task, as resistance can often be mediated by more than one mechanism in a single cell at the same time. Most of the earlier studies about taxane resistance focused on MDR and the development of novel taxanes was aimed at identifying compounds able to overcome it through different mechanisms of action. On the basis of numerous conventional SAR

studies and molecular modeling approaches, structural modifications have been introduced at different sites of paclitaxel or docetaxel, resulting in several series of new generation taxanes able to block multidrug transporters or to circumvent their action. In recent years, attention has been paid to the role of tubulin and microtubule alterations, which can affect the binding of taxanes to their target or counteract their stabilizing effects. As a result of the narrow period of time from the discovery of these alterations in paclitaxel-resistant cells and to the complexity of this kind of resistance, which can include several distinct mechanisms and can involve different proteins other than tubulin, the number of taxanes reported to be able to overcome it is quite restricted, and their mechanism of action at the molecular level has not yet been fully understood. Nevertheless, although we are far from an exhaustive comprehension of the resistance phenomenon, some of the newer taxanes developed in the last decade exhibited significantly improved activity profiles in comparison to paclitaxel and docetaxel, especially against resistant cancer cells, and a number of them are now undergoing clinical trials. A better understanding of the mechanisms involved in taxane resistance is expected to provide useful tools for the achievement of more selective treatments and for the rational design of novel versatile taxanes able to simultaneously overcome different resistance phenotypes.

Acknowledgements

Fondazione Monte dei Paschi di Siena is gratefully acknowledged for financial support.

Keywords: antitumor agents · drug resistance · microtubules · natural products · taxanes

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Received: December 22, 2006
Revised: February 23, 2007
Published online on May 25, 2007

