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Review Article

Novel tubulin-interacting agents: a tale of *Taxus brevifolia* and *Catharanthus roseus*-based drug discovery

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Abstract

Taxus brevifolia is the pacific yew tree from which paclitaxel (Taxol®) was first isolated. *Catharanthus roseus*, one of the most extensively studied medicinal plants in the world, has provided modern pharmacology with a large number of alkaloids, including the clinically important antitumor agents vinblastine and vincristine. Both classes of antitumor agents interfere with tubulin-microtubule dynamics through opposite modes of action. Considered to be excellent natural products, these drugs have been successfully brought to the market and served as leads for further optimization. This review highlights the chemical modification of *Taxus* diterpenoids and bisindole *Catharanthus* alkaloids, with emphasis on structure-activity relationship (SAR) studies and analogue optimization, which have culminated in the discovery of a new generation of antitumor drugs.

Introduction

Cancers and related diseases represent a major cause of mortality worldwide. With the development of cancer biology and pharmacology, great progress has been made toward a better understanding of cancerous diseases. Among current cancer treatment options, only chemotherapy can effectively treat systemic cancers. Such chemotherapy relies heavily on the use of natural products and their modified analogues. Vinblastine and paclitaxel (Taxol®) are among the most effective natural products for cancer chemotherapy known to date. They have been successfully brought to market and served as lead compounds for further optimization.

Microtubules are dynamic polymeric structures assembled from two structurally similar protein subunits, namely α- and β-tubulin. Each tubulin contains approximately 440 amino acid residues. Microtubules play an important role in cellular activities by providing pathways for cellular transport processes, constructing the cytoskeleton of cells and being involved in mitosis. Because cancer cells proliferate more rapidly than normal cells, disruption of cell division to stop the growth of diseased cells is clearly an important mode of action for many antitumor chemotherapeutic agents (1).

Mechanistically, there are two ways of disrupting the tubulin-microtubule equilibrium: 1) via the use of agents capable of promoting tubulin polymerization and stabilizing the microtubules thus formed from depolymerization (type 1); and 2) via the use of agents capable of preventing tubulin polymerization to produce microtubules (type 2).

Several representative tubulin polymerization promoters (type 1 compounds) are shown in Figure 1. These include paclitaxel **(1)**, epothilone B **(2)**, sarcodictyin A **(3)**, discodermolide **(4)**, eleutherobin **(5)**, peloruside A **(6)** and laulimalide **(7)** (2, 3).

A number of bisindole alkaloids derived from *Catharanthus roseus* (Fig. 2) were found to inhibit the tubulin polymerization process. *C. roseus* (L.) G. Don (Apocynaceae), also commonly known as rosy periwinkle, represents one of the most thoroughly examined plants in the world. Indigenous to Madagascar, *C. roseus* is cultivated in India, Israel and the U.S. (4). To date, more than 100 alkaloids have been isolated from the leaves and roots of this plant, including an extensive series of bisindole alkaloids (5). Of these, vinblastine **(8)** and vincristine **(9)** have become clinically important antitumor agents and are marketed as Velban® and Oncovin®, respectively. Vinblastine was first discovered

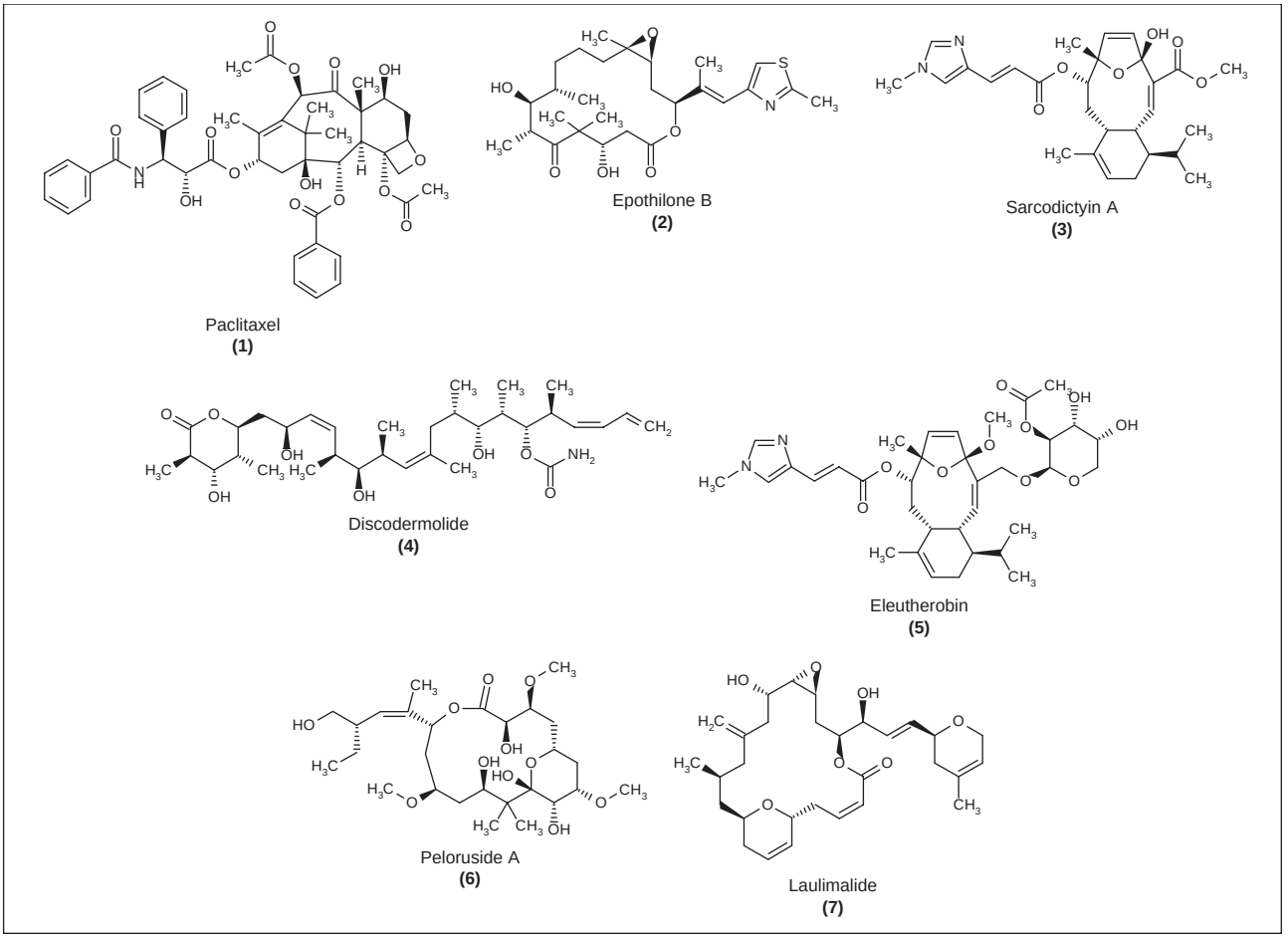


Fig. 1. Selected microtubule-stabilizing agents.

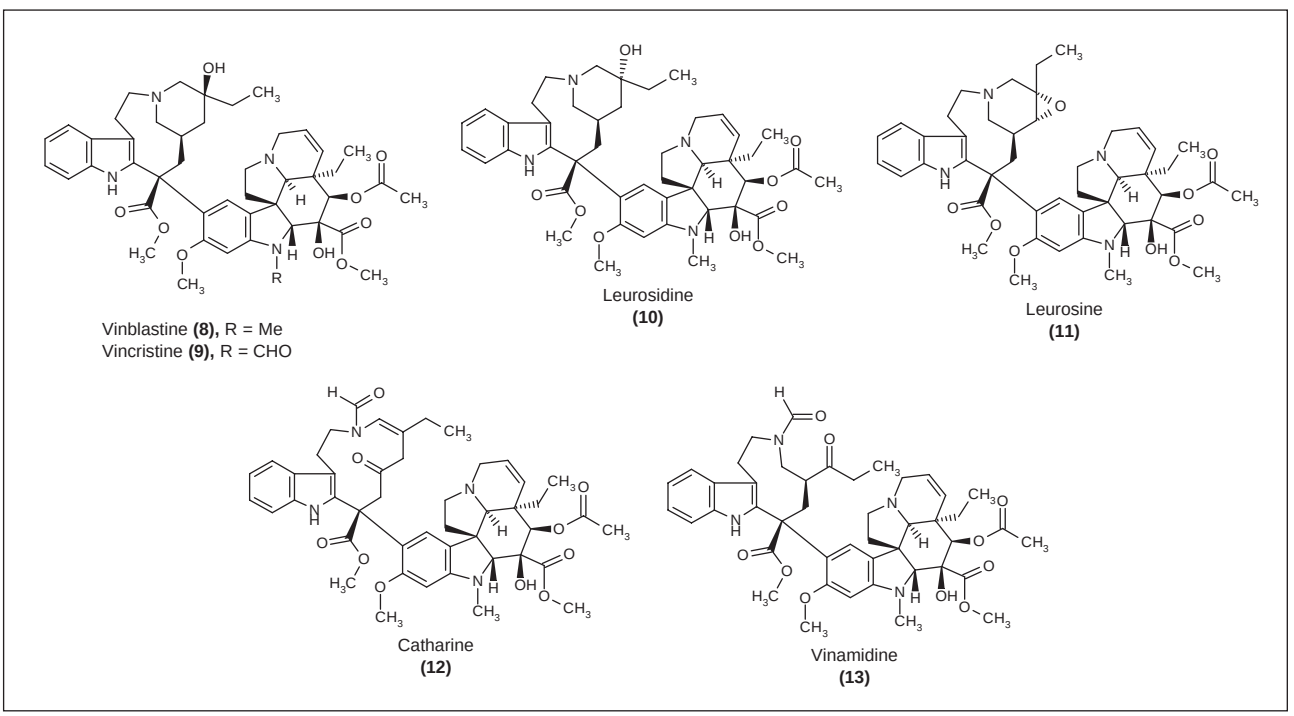


Fig. 2. Selected bisindole alkaloids from *Catharanthus roseus*.

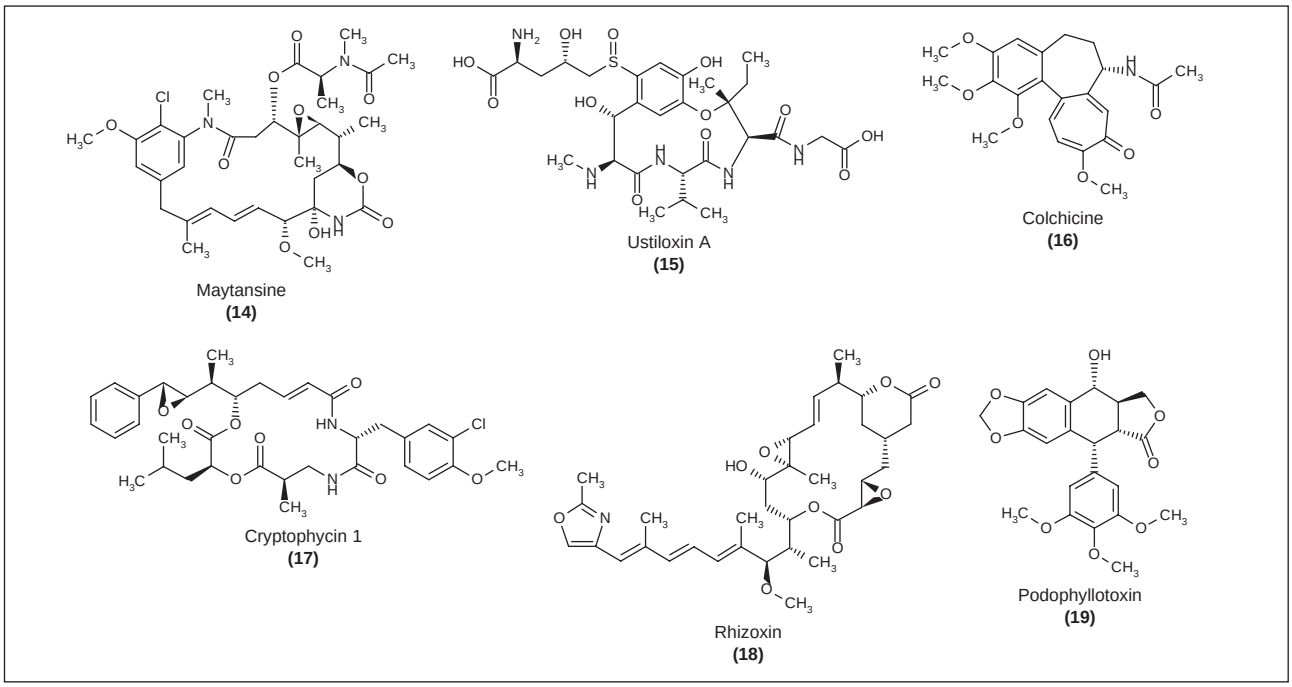


Fig. 3. Additional tubulin polymerization inhibitors.

in 1958 as an antiproliferative factor in leaf extracts of the periwinkle plant by Noble *et al.* at the University of Western Ontario (6, 7), and independently by Svoboda *et al.* at Lilly Research Laboratories (8, 9). Further purification of these extracts led to the discovery of vincristine (leurocristine), leurosidine **(10)**, leurosine **(11)**, catharine **(12)** and vinamidine **(13)** (10-12).

Other well-known tubulin polymerization inhibitors are shown in Figure 3 and include maytansine **(14)**, ustiloxin A **(15)**, colchicine **(16)**, cryptophycin 1 **(17)**, rhizoxin **(18)** and podophyllotoxin **(19)** (13). Like vinblastine and vincristine, they disrupt the cell division process primarily by destabilizing the structure of microtubules. Vinblastine and vincristine block the cell cycle at the G2/M phase. Like other chemotherapeutic agents, they exert their cytotoxic effects by interfering with the subtle interplay between tubulin and microtubules. The antineoplastic effects of vinblastine and vincristine are caused by the formation of a complex with tubulin (at a site different from that found with colchicine) to prevent tubulin polymerization, leading to inhibition of microtubule formation and blockade of mitosis (14).

Optimizing natural products from *Taxus brevifolia*

Paclitaxel **(1)**, an antimicrotubule agent initially isolated from the bark of *Taxus brevifolia* (15-18), has garnered considerable attention over the past 15 years due to its efficacy in the treatment of various types of cancer, including ovarian, breast and lung carcinoma (19). In addition, paclitaxel has demonstrated promising activity against Kaposi's sarcoma, bladder, prostate, esophageal, head and neck, cervical and endometrial cancers (20). Paclitaxel has a unique mechanism of action that distinguishes it from other anticancer agents. Its cytotoxic effects are believed to arise from its ability to promote tubulin polymerization and stabilize microtubules thus formed even in the absence of cofactors such as GTP. A major consequence of this shift in tubulin-microtubule equilibrium is the inhibition of mitosis (21).

The continually expanding therapeutic profile of paclitaxel, coupled with its novel mode of action, has spurred intense research activity on many fronts, including structure-activity relationship (SAR) studies (22, 23). A semisynthetic side-chain analogue of paclitaxel, docetaxel (Taxotere **[20]**), was approved by the FDA in 1996 for the treatment of advanced breast cancers (24, 25). Although both paclitaxel and docetaxel have demonstrated impressive antitumor activity, recent reports have indicated that the use of these drugs often results in undesirable side effects, as well as drug resistance (26). Therefore, it is necessary to develop better taxanes with improved efficacy and safety profiles against paclitaxel-sensitive and -resistant tumors. Furthermore, it is desirable to deliver these novel taxanes via oral formulations.

With these considerations in mind, many research groups have engaged in the design and synthesis of novel taxanes in the hope of discovering superior paclitaxel analogues via both core and side-chain modifications. Results emerging from rather extensive SAR studies at the diterpenoid core clearly show that chemical modifications of the functional groups at the northern region of the molecule, namely C-7 (27), C-9 (28-30) and C-10 (31, 32), as well as those located at the southern region of paclitaxel, namely C-2 (33-41), the oxetane ring (42-46), C-4 (47-52) and C-1 (43, 53), could impact the

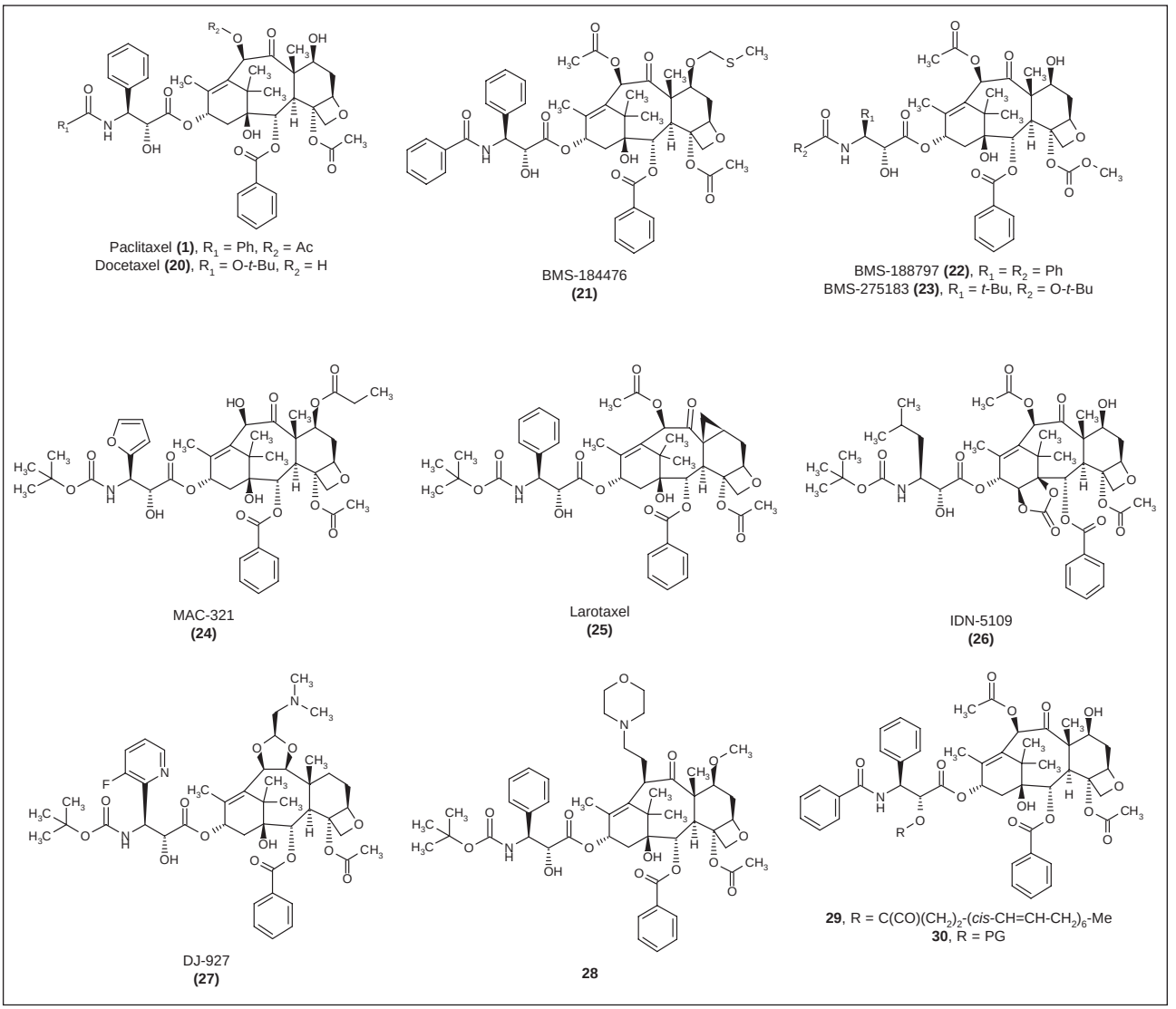


Fig. 4. Newly discovered promising paclitaxel derivatives.

tubulin binding affinity and cytotoxicity of the newly designed paclitaxel derivatives.

As a result of fruitful research conducted at both academic institutions and in the pharmaceutical industry, a number of “better” paclitaxel analogues incorporating side-chain and/or core modifications were discovered and have been advanced to various stages of clinical trials (Fig. 4). These include three core-modified paclitaxel analogues from Bristol-Myers Squibb, namely BMS-184476 **(21)** (54-58), BMS-188797 **(22)** (43, 58-63) and BMS-275183 **(23)** (64-68). In addition, two C-7-modified analogues bearing a docetaxel side-chain, MAC-321 **(24)** (69) and larotaxel **(25)** (70-73), along with the C-14-hydroxylated paclitaxel analogue IDN-5109 (SB-T-101131 **[26])** (74-77) with additional modifications on its side-chain, were selected for clinical trials. More recently, two C-10-modified paclitaxel derivatives, DJ-927 (tesetaxel **[27]**) (78-82) and compound **28** (83-85), discovered at Daiichi Pharmaceutical (now Daiichi Sankyo), demonstrated antitumor efficacy following oral administration. In addition, two water-soluble paclitaxel prodrugs **(29, 30)** were also selected for clinical evaluation (86, 87).

In this review, we intend to focus on the SAR trends observed with the functionalities located at the southern part of the taxane scaffold, namely C-2, C-4 and the oxetane (D-ring) region. In particular, we will present detailed information from the tubulin polymerization assay, cytotoxicity data as determined in various paclitaxel-sensitive and paclitaxel-resistant cell lines, and antitumor activity profiles for selected C-4 analogues. Furthermore, we will briefly highlight the SAR progression that led to the discovery of the C-4-modified second-generation paclitaxel analogue BMS-188797 **(22)** (43, 58-63). Continuing side-chain SAR exploration of BMS-188797 led to the discovery of BMS-275183 **(23)**, another C-4-modified paclitaxel analogue with oral activity (64-68). Finally, this review will

also include a brief update of recent developments concerning several novel taxanes, including BMS-184476, MAC-321, IDN-5109, DJ-927 and compound **28**.

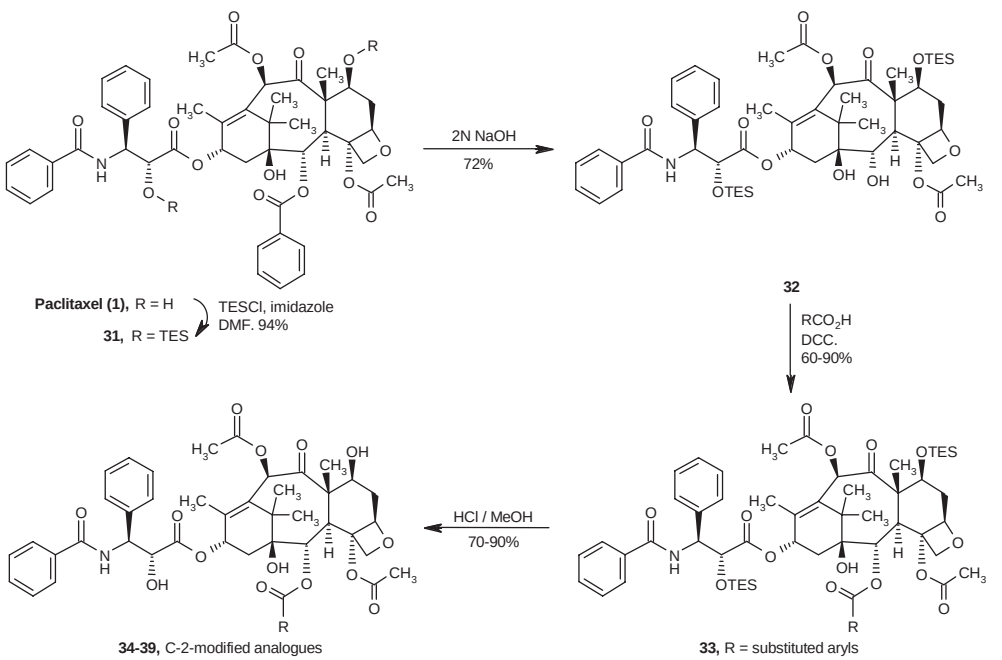
C-2 SAR investigation

A convergent synthetic route for the preparation of C-2-modified paclitaxel analogues was reported (35). As outlined in Scheme 1, the C-2 and C-7 bis-silylated paclitaxel **(31)** was subjected to debenzoylation under phase-transfer conditions to yield the corresponding C-2 hydroxyl derivative **(32)**. This intermediate was then condensed with the requisite C-2 acids to provide the respective C-2 ester derivatives **(33)**, which were converted to the final products **(34-39)** upon desilylation (Fig. 5).

In 1993, Chen *et al.* reported that the C-2 deoxypaclitaxel **(39)** was > 100-fold less cytotoxic than paclitaxel against human colon cancer HCT 116 cells, suggesting that the C-2 benzoate was involved in the tight binding of paclitaxel to its receptor (33). Subsequent reports (34-36) disclosed the following IC_{50} values for cytotoxicity in HCT 116 cells, as determined for a set of C-2-substituted benzoates: IC_{50} > 88 nM for **34**; IC_{50} = 0.70 nM for **35**; IC_{50} = 3.8 nM for **36**; IC_{50} = 0.36 nM for **37**; and IC_{50} = 2.8 nM for **38**. Compared to paclitaxel (IC_{50} = 2-2.5 nM), **35** and **37** were found to be more potent (36). In view of the poor biological activity detected for **34**, it is clear that *para*-substitution was not tolerated. A separate report demonstrated that, on the basis of the cytotoxicity determined in B16 melanoma cell lines, the C-2 phenyl-bearing analogue **(40)** was about 5-fold more potent than its corresponding C-2-saturated counterpart **(41)** (37). These results are in good agreement with those disclosed by Duclos *et al.* on corresponding paclitaxel analogues (38). More recently, Kadow *et al.* prepared a series of structurally unique C-1/C-2 orthoester derivatives, including **42** (Fig. 5). Interestingly, this novel analogue retained good cytotoxicity against HCT 116 cells, with an IC_{50} value of 6 nM (39). It is also worth mentioning that the C-2 *epi*-paclitaxel **(43)** was devoid of bioactivity in both the tubulin binding assay and the cytotoxicity assay when tested against the HCT 116 cell line (40). More recently, Fang *et al.* showed that the C-2 benzamide-bearing derivative **(44)** was 16-fold less cytotoxic than paclitaxel in KB cells (41). These results provide further evidence to support the importance of the nature and stereochemistry of the C-2 functionality for the bioactivity of paclitaxel (40, 41).

Encouraged by the excellent potencies determined in the paclitaxel-sensitive HCT 116 cell line, a number of promising C-2 analogues were further evaluated in the paclitaxel-resistant cell line 1A9 (PTX22). This cell line expresses an altered β-tubulin that confers 21-fold resistance to paclitaxel. When tested against the 1A9 (PTX22) cell line, compounds **36** and **37** showed 15-fold resistance relative to the corresponding sensitive cell line. In contrast to paclitaxel, the C-2 *meta*-azidobenzoate derivative **(38)** was associated with only 5-fold resistance (36). These results clearly demonstrated that SAR modification of the C-2 benzoate functionality could exert a marked influence on the resistance profile of taxane derivatives.

Scheme 1: Synthesis of C-2-modified paclitaxel analogues



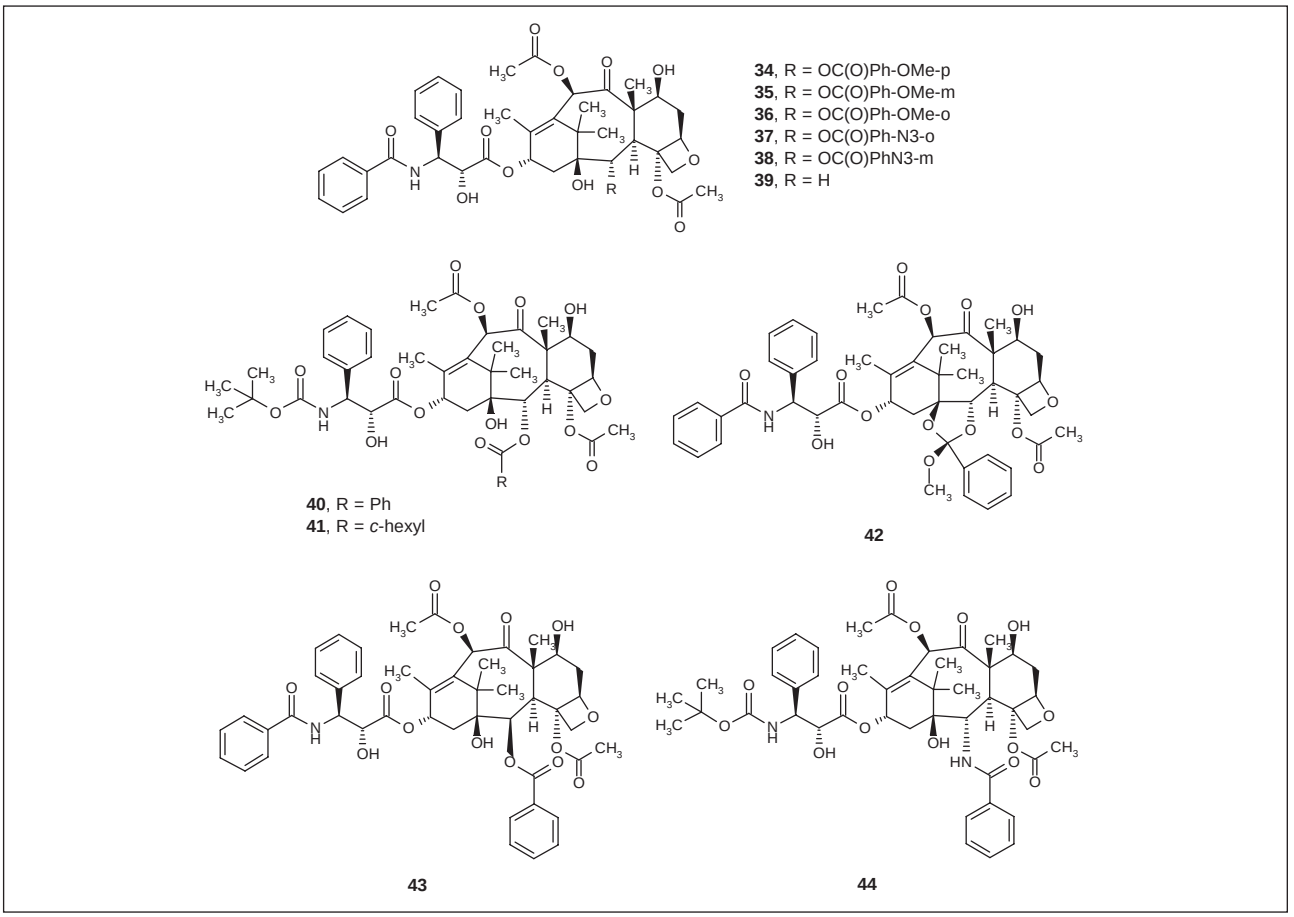


Fig. 5. Representative C-2-modified paclitaxel analogues.

Oxetane ring modification

In order to probe the importance of the intact oxetane ring for bioactivity, several oxetane ring-opened analogues (*e.g.*, **45** and **46**) and oxetane ring oxygen-modified analogues (*e.g.*, **48** and **49**) were prepared and evaluated for their tubulin assembly activity and *in vitro* cytotoxicity against cancer cell lines (Fig. 6).

Pioneering work by Samaranayake *et al.* showed that a D-secotaxol derivative such as **45** was devoid of biological activity (42). On the basis of this result, it was concluded that the oxetane ring is essential for the interaction with microtubules and for cytotoxicity. Since the key C-4 acetate moiety was absent in **45**, the search continued for compounds bearing both the intact C-4 functionality and the modified oxetane ring. In 1993, Chen *et al.* prepared an interesting bicyclic D-ring-containing analogue **(46)** with the intact C-4 acetate seen in paclitaxel. Surprisingly, **46** also showed significantly reduced bioactivity relative to paclitaxel (43). The lack of bioactivity observed with **46** seemed to suggest that the oxetane ring is required for activity. A report from Marder-Karsenti *et al*. detailed the synthesis and evaluation of two 5(20)-aza-docetaxel derivatives. When tested against the KB cell line *in vitro*, both **47** and **48** were found to be inactive. In addition, compound **47** showed 16-fold weaker potency relative to docetaxel, and compound **48** was essentially inactive in the tubulin polymerization assay (44). The nitrogen atom in the azetidine ring (D-ring) would be protonated at neutral pH and thus would possibly interact with tubulin at a different site than that found with the neutral oxygen atom in docetaxel. At this point in the investigation, the role of the oxetane ring on the activity of paclitaxel or docetaxel was still not well understood. Therefore, in order to pinpoint the role of the oxetane-ring oxygen atom on activity, Gunatilaka *et al*. embarked on the synthesis and comparative evaluation of the 5(20)-thia-paclitaxel derivative **(49)** (45) and its corresponding C-4 methylcarbonate paclitaxel analogue BMS-188797 **(22)**. When tested in the tubulin polymerization and cytotoxicity assays, compound **49** had negligible activity in all assays, with IC_{50} values of > 1 μM for Burkitt's lymphoma and > 2.5 μM for prostate carcinoma. In contrast, BMS-188797 was clearly superior in activity to paclitaxel and even to docetaxel. The IC_{50} values determined for BMS-188797 were 2 nM for CA46 Burkitt's lymphoma, 1 nM for PC-3 prostate carcinoma and 0.2 nM for MCF7 breast carcinoma. On the basis of these results, the researchers concluded that the D-ring region of the paclitaxel pharmacophore is very sensitive to steric effects and that the oxygen atom in the

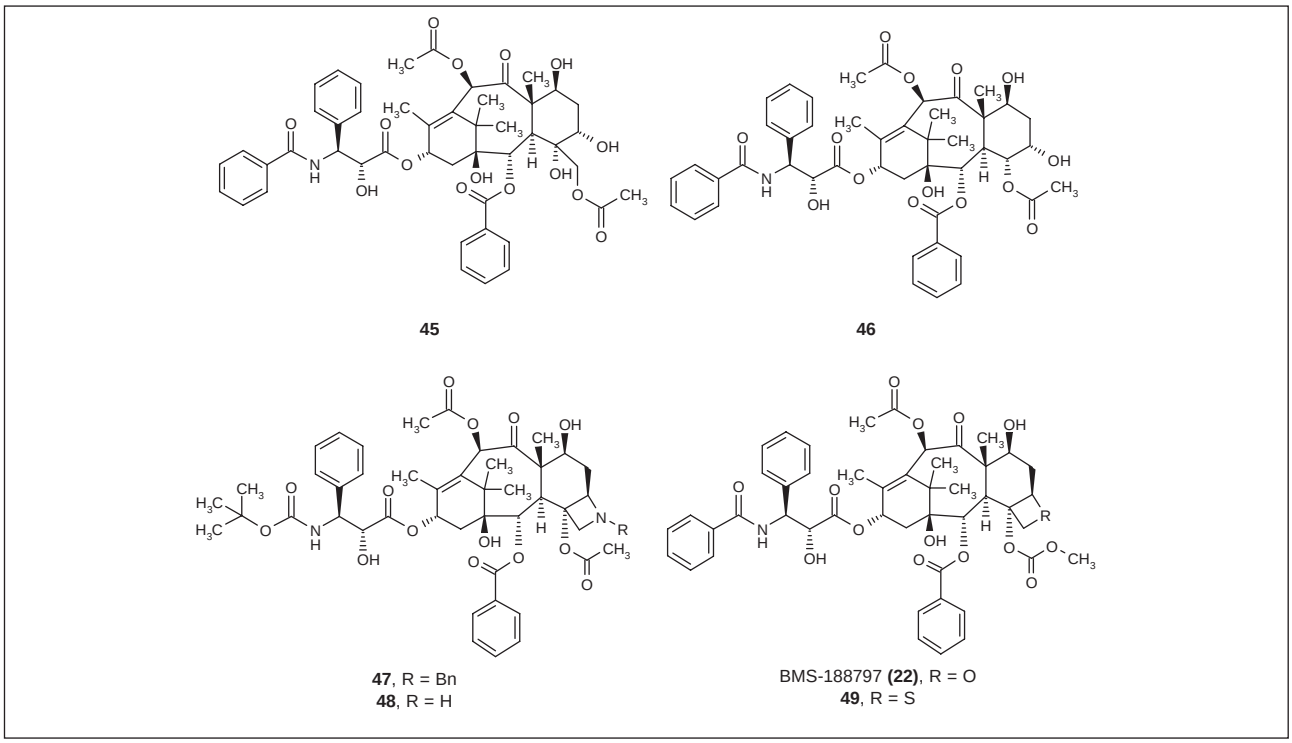


Fig. 6. Oxetane ring-modified paclitaxel and docetaxel derivatives.

oxetane ring is acting as a hydrogen bond acceptor (45). A similar 5(20)-thia-docetaxel derivative was later prepared by Merckle *et al*. (46). Taking all the data discussed here as a whole, it is evident that either opening the oxetane ring or replacing the oxygen atom within the oxetane ring with nitrogen or sulfur leads to paclitaxel analogues with reduced cytotoxicity.

C-4 SAR investigation

As shown in Scheme 2, two novel series of C-4 ester and carbonate paclitaxel analogues were synthesized from the key intermediate 4-deacetyl-baccatin derivative **(52)**, which was in turn prepared according to Chen's protocol (Red-Al/THF/0°C) in two steps from 7,13-bis-TES-baccatin III **(50)** (47). Deprotonation of **52** with lithium bis(trimethylsilyl)amide, followed by reaction with an acyl chloride or a chloroformate, resulted in the desired C-4 ester baccatin **(53)** or the C-4 carbonate baccatin **(54)**, with a 42-94% yield. These two intermediates were converted, via desilylation (at C-1, C-7 and C-13) and subsequent monoresilylation, to their corresponding 7-TES-baccatin derivatives (**55** and **56**, respectively). Final side-chain attachment (at C-13) onto **55** or **56** led to two novel series of C-4-modified paclitaxel analogues (**58** and **59**, respectively) (47).

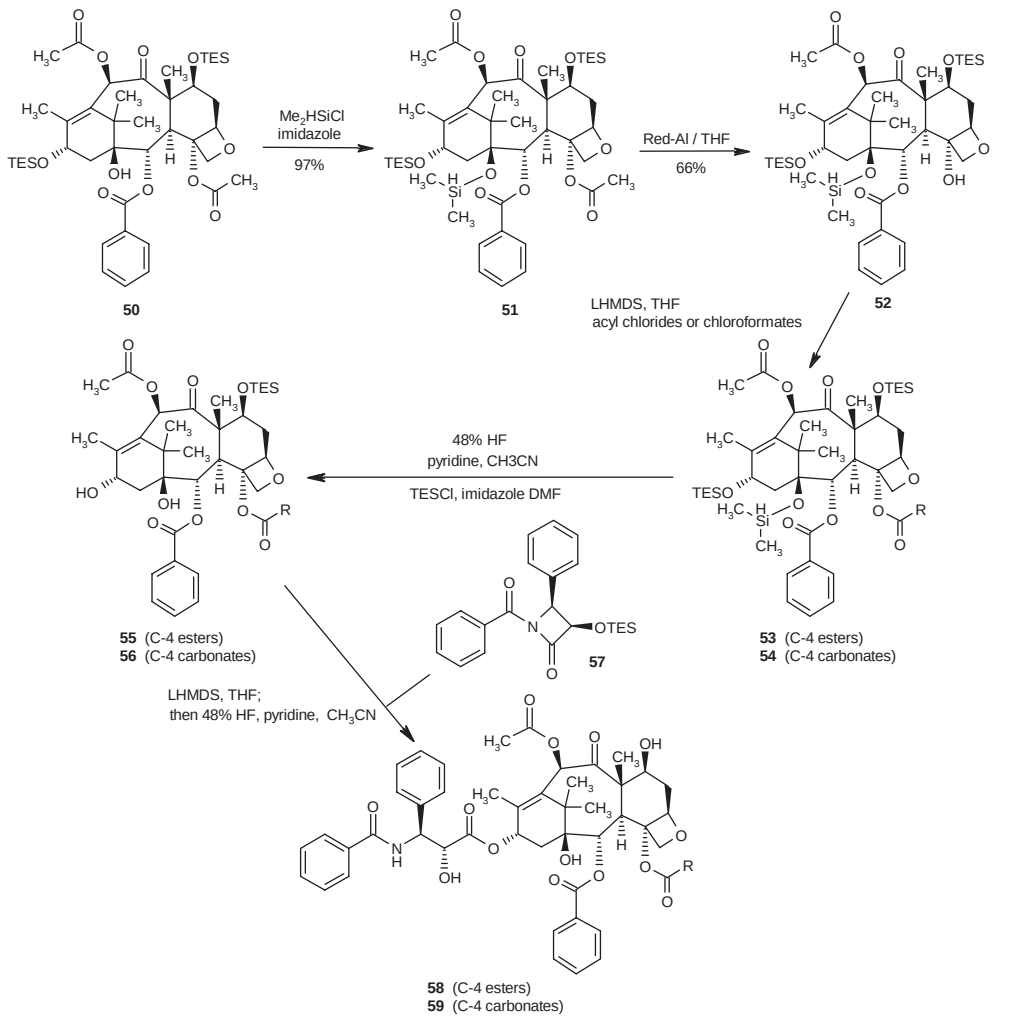
As shown in Table I, the C-4 deacetyl derivative **(63)** (Fig. 7) was considerably less potent than paclitaxel in the tubulin polymerization and cytotoxicity assays (48, 51). In 1996, Chen *et al*. reported that the C-4 methyl ether analogue **(64)** displayed 18-fold weaker cytotoxicity in comparison to paclitaxel (50). These results clearly indicated that the C-4 acyl moiety is an important element in receptor binding. To further explore the size of the C-4 binding pocket, Chen *et al*. synthesized and evaluated a large number of C-4 analogues, including various C-4 esters and C-4 carbonates, as highlighted in Table I. Interestingly, two C-4 benzoyl-bearing derivatives **(65, 66)** were significantly less active than paclitaxel, suggesting that the binding pocket at C-4 could not accommodate aromatic rings (48).

In sharp contrast to the results mentioned above, all of the aliphatic esters **(67-71)** exhibited good to excellent activity in the tubulin and cytotoxicity assays. A rather important trend can be observed by simply comparing the cytotoxicity data for the following five C-4 straight-chain (2-6 carbons) ester analogues: paclitaxel, **67-69** and **71**. It is obvious that the 4-butyrate ester **(68)** was the most potent analogue within this subset. This trend also indicates that the 4-carbon chain is probably the optimal size for effective receptor binding.

In order to further optimize activity, other 4-carbon-bearing C-4 esters, including the C-4 cyclopropyl ester analogue **(60)**, were prepared. To our satisfaction, **60** was found to be the most potent of the ester derivatives presented in Table I (47, 48). A similar trend was also found with the C-4 cyclobutyl-bearing analogue **(70)**, which showed improved activity in the tubulin assay in comparison to **69**.

Interestingly, the C-4 aziridine carbamate derivative **62** (Fig. 7), a closely related analogue of the C-4 ester **60**, was found to be 10-fold less potent than **60** in the tubulin

Scheme 2: Synthesis of C-4 ester and carbonate paclitaxel derivatives



polymerization assay (49). As shown in Table I, two C-4 carbonate analogues (BMS-188797, **61**) exhibited 2-4-fold improved potency relative to paclitaxel in both the tubulin binding assay and the cytotoxicity assay against the HCT 116 cell line (48).

Novel side-chain-modified C-4 esters and carbonates: discovery of BMS-188797

In light of recent reports indicating that replacement of the 3'-C-phenyl and 3'-N-Bz with their respective 3'-C-furyl and 3'-N-Boc could lead to paclitaxel side-chain analogues possessing enhanced *in vitro* activity (88-94), several potent C-4 esters **(60, 68)** and carbonates (BMS-188797, **61**) were further derived to their side-chain analogues for biological evaluation. The *in vitro* cytotoxicities against sensitive HCT 116 and multidrug-resistant HCT 116 (VM46) cells and *in vivo* efficacies (i.p.) of these analogues are summarized in Table II.

The general conclusion that emerges from the *in vitro* data is that side-chain modifications do not seem to have a significant impact on the cytotoxicity of the C-4 esters and C-4 carbonates listed in Table II. In fact, none of the 3'-C and/or 3'-N replacements produced a clear benefit over the corresponding parent compounds. For example, four C-4 cyclopropyl esters **(60, 73-75)** and two C-4 methylcarbonates (BMS-188797, **76**) displayed almost equal potencies in the HCT 116 cytotoxicity assay, regardless of their side-chain substituents (43).

Careful analysis of the R/S ratio listed in Table II clearly indicates that a moderate increase in size at C-4 resulted in an improved ability to overcome resistance in the HCT 116 (VM46) cell line (*e.g.*, **60, 72-77**). This cell line is over 100-fold resistant to paclitaxel due to the overexpression of P-glycoprotein (P-gp), which results in decreased intracellular concentrations of paclitaxel and leads to the multidrug-resistant (MDR) phenotype. Although clinical resistance to paclitaxel is not well under-

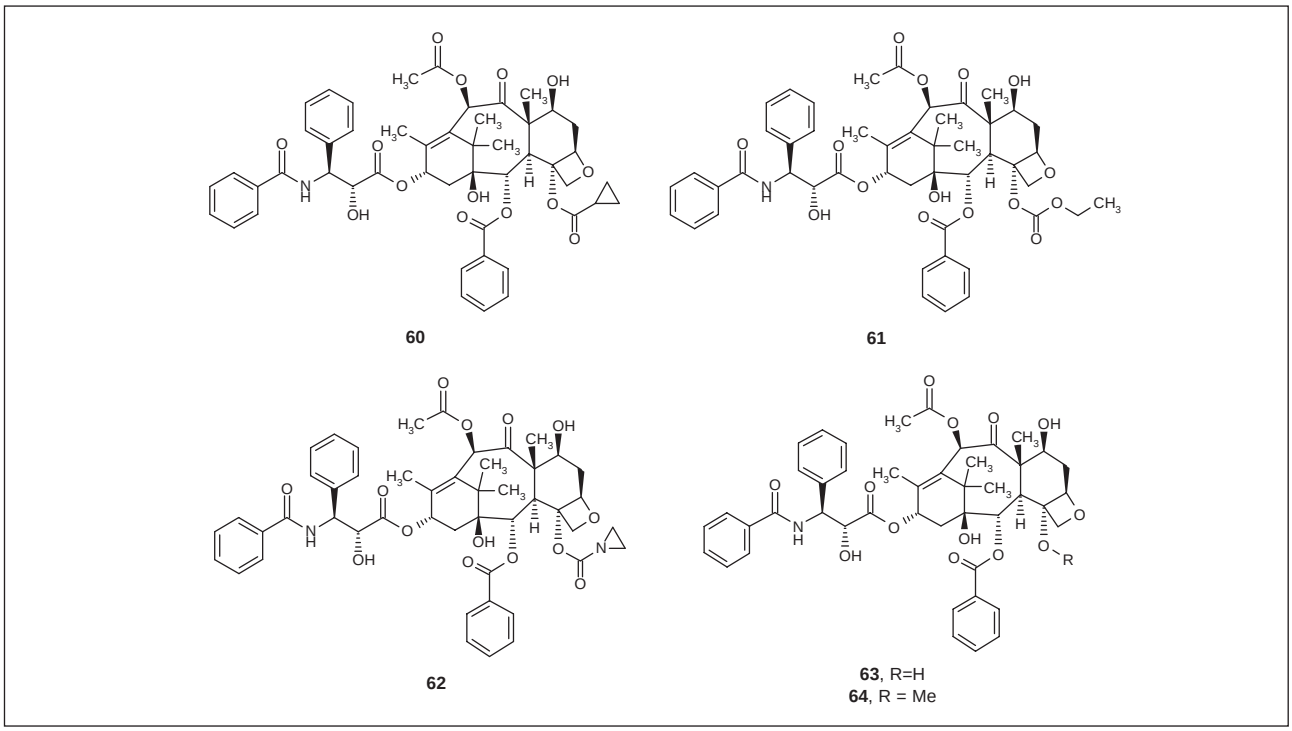


Fig. 7. Representative C-4-modified paclitaxel analogues.

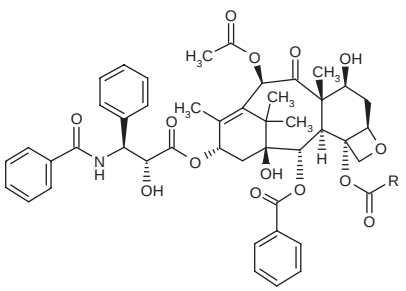


Table I: In vitro potencies of paclitaxel side-chain-bearing C-4 analogues.

Analogue	R	Tubulin polymerization ratio[1]	HCT 116 IC_{50} (nM)
Paclitaxel **(1)**	Me	1.0	2.4-4.0
65	Ph	> 60	> 1000
66	p-F-Ph	61	790
67	Et	1.5	2.0
68	*n*-Pr	0.61	1.1
60	*c*-Pr	0.24	1.0
69	*n*-Bu	1.0	2.0
70	*c*-Bu	0.44	1.5
71	$(CH_2)_4CH_3$	3.4	6.0
BMS-188797 **(22)**	OMe	0.41	2.0
61	OEt	0.64	1.0

[1]Ratio obtained in the tubulin polymerization assay, with ratios of < 1.0 indicating analogues that were more potent than paclitaxel.

stood, P-gp overexpression and altered tubulin have been suggested as possible mechanisms. Remarkably, as shown in Table II, replacement of the C-4 acetoxy moiety in paclitaxel with either cyclopropyl ester **(60)** or ethylcarbonate **(61)** effectively overcame P-gp-mediated resistance to paclitaxel by a factor of 2.4 or 2.9, respectively. In these cases, further side-chain modifications did not greatly impact the R/S ratio (see data obtained for **73-75, 77**). On the other hand, for other C-4 analogues (BMS-188797, **68**), such side-chain modifications (*t*-Boc as the

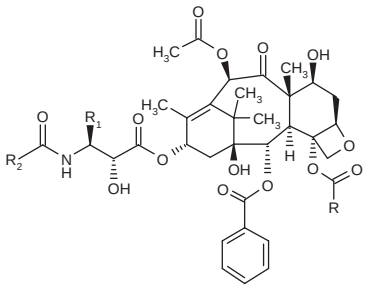


Table II: In vitro and in vivo activity of paclitaxel side-chain analogues.

Analogue	R	R_1	R_2	HCT 116 IC_{50} (nM)	R/S Ratio[1]	M-109[2] T/C (mg/kg i.p.)
Paclitaxel **(1)**	Me	Ph	Ph	2.4-4.0	125	159-228 (60)
68	*n*-Pr	Ph	Ph	1.1	31	164 (25)
72	*n*-Pr	2-Furyl	t-Boc	2.8	0.5	96 (1)
60	*c*-Pr	Ph	Ph	1.0	2.4	188 (6)
73	*c*-Pr	Ph	t-Boc	0.8	0.9	115 (1.6)
74	*c*-Pr	2-Furyl	Ph	0.9	5.2	132 (3)
75	*c*-Pr	2-Furyl	t-Boc	1.3	1.3	100 (4)
BMS-188797 **(22)**	OMe	Ph	Ph	2.0	68	161 (50)
76	OMe	2-Furyl	t-Boc	1.6	3.6	183 (32)
61	OEt	Ph	Ph	1.0	2.9	142 (6)
77	OEt	2-Furyl	t-Boc	3.0	1.4	144 (4)

[1]R/S ratio defined as IC_{50} for multidrug-resistant (MDR) HCT 116 cell line/IC_{50} for sensitive HCT 116 cell line. [2]M-109 Madison murine lung carcinoma.

3'-N cap and/or 2-furyl as the 3'-C replacement) had a significant impact on the R/S ratio. The resulting non-paclitaxel side-chain analogues (**72** and **76**) exhibited impressive R/S ratios ranging from 0.5 to 3.6 (60).

As shown in Table II, of the 10 C-4 esters and carbonates tested against murine M-109 lung carcinoma (23, 26), 7 were active (T/C > 125%). The best results were obtained with the paclitaxel side-chain-bearing 4-cyclopropyl ester **(60)** and the non-paclitaxel side-chain-containing 4-methylcarbonate **(76)**, which had T/C values of 188% and 183%, respectively. Some of these C-4 analogues were 10-15 times more potent than paclitaxel (according to mg/kg dose values). These include two C-4 cyclopropyl esters **(60, 74)** and two C-4 ethylcarbonate derivatives (61, 77). However, attempts to improve the *in vivo* efficacy via further side-chain modifications were not successful. For instance, 3'-C-furyl/3'-N-Boc-bearing C-4 cyclopropyl analogues **(73-75)** and the C-4 *n*-propyl analogue **(72)** possessed reduced activities (as measured by T/C values) compared to their corresponding parent analogues (**60** and **68**). In one case, the C-4 methylcarbonate **(76)** exhibited slightly better *in vivo* activity than the paclitaxel side-chain-bearing analogue BMS-188797. No side-chain effect was seen with C-4 ethylcarbonate analogues (**61** *vs*. **77**). In view of these discrepancies, it is fair to say that side-chain modification provided at best only minimal improvements in *in vivo* efficacy in the M-109 (i.p.) tumor model (48, 60).

Promising C-4 esters and carbonates with demonstrated *in vivo* efficacy in the M-109 (i.p.) solid tumor model were tested in one or more secondary distal tumor models (i.v. administration of drug to mice bearing a tumor implanted s.c.) in an effort to identify better paclitaxel derivatives. Disappointingly, none of these compounds was found to be more effective than paclitaxel. It became apparent in the C-4 analogue series that an enhanced ability to overcome MDR *in vitro* did not correlate with enhanced *in vivo* efficacy. This observation parallels the results of Nicolaou's group obtained with C-2 taxoids (92), as well as our own findings with C-7 paclitaxel derivatives (27). Despite the limitations seen with the initial i.p./i.p. M-109 tumor model, the C-4 cyclopropyl ester **(60)** and the C-4 methylcarbonate derivative BMS-188797 demonstrated the best activities. In light of the fact that furyl/Boc side-chain analogues in the C-4 cyclopropyl series **(73, 75)** were inactive *in vivo*, it seemed possible that the *in vivo* activity of the C-4 methylcarbonate series might be more robust in tolerating the incorporation of a more potent side-chain. To confirm this hypothesis, the C-4 methylcarbonate BMS-188797 was further evaluated in distal tumor models (against L2987 lung carcinoma and HCT/pk colon carcinoma) despite its modest ability to overcome resistance *in vitro*. To our satisfaction, BMS-188797 demonstrated better efficacy than paclitaxel in these distal tumor models after i.v. administration (95). On the basis of these data, BMS-188797 was selected for further evaluation in phase I and II clinical trials.

Results from a phase I study and a pharmacokinetic study of BMS-188797 were reported recently by Advani *et al*. (61) and Garrett *et al*. (62), respectively. In the first

study, a total of 18 patients with advanced malignancies were treated with escalating doses of BMS-188797 at 35 (n=3), 50 (n=9) and 65 (n=6) mg/m^2 on a weekly schedule via 1-h i.v. infusion. Mean plasma C_{max} and $AUC_{0\text{-}48h}$ increased in a dose-dependent manner within the range of doses used in this study. In 3 of 4 patients, the dose-limiting toxicities (DLTs) correlated with the AUC. Two partial responses (lung cancer, 7+ months; ovarian cancer, 6+ months) and 2 minor responses (esophageal cancer, 5 months; ovarian cancer, 5 months) were obtained. It is worth mentioning that the 2 patients with partial responses had been clinically resistant to paclitaxel. In light of these results, it was concluded that BMS-188797 demonstrated antitumor activity in taxane-refractory solid tumors. Based on the findings from this phase I study, the MTD and the recommended phase II dose of BMS-188797 was estimated to be 50 mg/m^2. This drug is also being evaluated in combination with carboplatin (61).

In a separate phase I study, a total of 51 patients were treated with a 1-h i.v. infusion of BMS-188797 once every 21 days for a total of 160 cycles of therapy (62). The initial dose of 3.75 mg/m^2 was set at approximately one-third of the lethal dose in dogs. The DLT of febrile neutropenia occurred in 2 patients at 200 mg/m^2. Four partial responses lasting 24.1 months (renal cell carcinoma), 5.7 and 4.3 months (breast cancer) and 4.5 months (non-small cell lung cancer) were obtained in this study. Pharmacokinetics were found to be linear at doses through 110 mg/m^2, but not at higher doses. On the basis of the pharmacokinetic data observed in this study, the recommended phase II dose of BMS-188797 as a single agent was 175 mg/m^2 by 1-h infusion every 3 weeks (62).

BMS-188797-based side-chain modifications: discovery of the orally active taxane BMS-275183

With the aim of identifying orally active paclitaxel derivatives, scientists from Bristol-Myers Squibb embarked on a screening of their paclitaxel analogue inventory using a murine oral exposure assay. Interestingly, BMS-188797 was identified as the most promising lead from this effort. As outlined in Table III, BMS-188797 showed 46-fold higher oral exposure relative to paclitaxel. However, in a subsequent definitive study using crystalline material, BMS-188797 was found to possess minimal oral bioavailability (4%). Therefore, additional BMS-188797-based side-chain-modified analogues were prepared and evaluated in the search for novel taxanes endowed with oral efficacy and with potent cytotoxicity against both sensitive and resistant HCT 116 cell lines. Compounds that showed higher drug plasma levels than BMS-188797 after oral dosing were evaluated further for oral efficacy in the M-109 murine lung tumor model. The compounds with a demonstrated log cell kill (LCK) ratio between 0.8 and 1.2 (orally dosed analogue *vs*. i.v. paclitaxel) were selected for additional *in vivo* testing (66).

At the onset of this program, a series of 3'-N-modified analogues were synthesized. As shown in Table III, the C-3'-Boc-protected analogue **(78)** was found to be 7-fold more potent and exhibited 3-fold lower resistance in the MDR HCT 116 cell line as compared to BMS-188797. Moreover, compound **78** showed 4-fold higher oral exposure than that achieved by BMS-188797. Unfortunately, compound **78** did not demonstrate oral efficacy equivalent to that of i.v. paclitaxel. Continuing C-3'-N modifications led to analogues **79** and **80**, both of which displayed comparable *in vitro* cytotoxicity but higher oral exposure levels relative to BMS-188797. Furthermore, both **79** and **80** demonstrated oral efficacy comparable to i.v. paclitaxel in the M-109 tumor model (66).

Further compound **78**-based C-3' modifications yielded the *t*-butyl-bearing analogue BMS-275183 **(23)** and the isopropyl-bearing analogue **81** (Table III). Although both BMS-275183 and **81** were found to be 2-5-fold less cytotoxic than **78**, these C-3'-modified analogues exhibited an improved (by 3-5-fold) R/S ratio in comparison to **78**. In the subsequent oral exposure study, BMS-275183 and compound **81** achieved about 2-fold higher plasma levels than BMS-188797. Furthermore, oral administration of these analogues demonstrated antitumor activity similar to that of i.v. paclitaxel in the M-109 model. Replacement of the 3'-N-Boc moiety in BMS-275183 with neopentyl amide yielded **82**, which was found to be 7- and 4-fold less cytotoxic than BMS-275183 and compound **80**, respectively, when tested against a sensitive HCT 116 cell line. The R/S ratio observed with **82** was also lower than that detected with BMS-275183 or compound **80**. Continuing 3'-N modification of BMS-275183 afforded two analogues **(83, 84)** that were about 3.5-fold less cytotoxic. Interestingly, these analogues showed 2-fold higher oral exposure than BMS-275183. When tested in the M-109 model after oral administration, compound **84** achieved the same activity as i.v. paclitaxel (66).

Those analogues with good oral efficacy in the M-109 model were selected for further evaluation in the A2780 human ovarian carcinoma xenograft model. BMS-275183 and compound **81**, with an alkyl moiety incorporated at the C-3' position, continued to perform well in this experiment, achieving an LCK of 4.4 (at 65 mg/kg) and 3.0 (at 28 mg/kg), respectively. Two C-3' phenyl-bearing analogues **(79, 80)** also demonstrated good efficacy, with LCK values of 7.0 and 3.8, respectively, achieved when dosed at 100 mg/kg. Two 3'-*N*-modified analogues **(82, 84)** achieved an LCK of 1.8 (at 36 mg/kg) and 2.1 (at 120 mg/kg), respectively. Of these 6 compounds, the C-3' *t*-Bu-bearing analogue BMS-275183 showed the highest oral bioavailability in rats, as well as an excellent solubility profile (16.5 mg/ml in 10:10:80 Cremophor EL:ethanol:water) (66).

In view of the exciting data generated, BMS-275183 was selected for further evaluation in additional tumor models. Extensive evaluation of BMS-275183 demonstrated antitumor activity equal to that of i.v. paclitaxel in C3H mammary carcinoma and HCT/pk colon carcinoma. This orally active taxane also showed activity in the CWR-22 human hormone-dependent prostate tumor model.

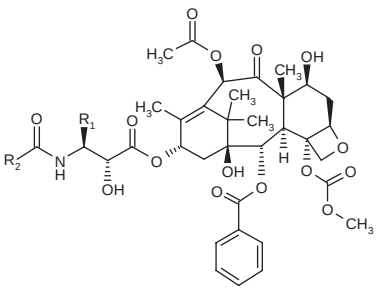


Table III: BMS-188797-based side-chain modifications.

Analogue	R1	R2	Tubulin polymerization ratio[1]	HCT 116 IC_{50} (nM) (R/S ratio)[2]	Murine oral absorption (ng/ml)[3]	M-109 ratio[4] (mg/kg p.o.)
Paclitaxel **(1)**	Ph	Ph	1.0	4.0 (114)	84	0.02 (160)
BMS-188797 **(22)**	Ph	Ph	0.4	2.3 (68)	3,935	0.4 (32)
78	Ph	*t*-Butoxy	—	0.33 (22)	15,626	0.6 (15)
79	Ph	*n*-Butoxy	0.9	1.3 (24)	6,641	0.9 (50)
80	Ph	Neopentyl	0.9	3.6 (24)	7,877	1.3 (50)
BMS-275183 **(23)**	*t*-Bu	*t*-Butoxy	0.4	1.8 (3.9)	7,636	0.8 (25)
81	*i*-Pr	*t*-Butoxy	0.9	0.53 (7.1)	7,205	0.9 (12)
82	*t*-Bu	Neopentyl	0.9	14 (84)	8,087	1.0 (160)
83	*t*-Bu	*i*-Butyl	0.6	6.1 (6)	16,853	0.6 (100)
84	*t*-Bu	Cyclobutyl	0.8	7.0 (> 17)	18,223	1.1 (60)

[1]Ratio obtained in the tubulin polymerization assay, with ratios of < 1.0 indicating analogues that were more potent than paclitaxel. [2]R/S ratio defined as IC_{50} for MDR HCT 116 cell line/IC_{50} for sensitive HCT 116 cell line. [3]Mouse plasma concentration of the analogue 1 h after oral dosing at 100 mg/kg in 10:10:80 Cremophor EL:ethanol:water. [4]M-109 Madison murine lung carcinoma tumors implanted s.c. Values presented are a ratio of the log cell kill (LCK) for the analogue at MTD *versus* LCK for i.v. paclitaxel.

When evaluated in combination with paclitaxel, therapeutic advantages were evident for tumor-bearing mice that received BMS-275183 either after induction chemotherapy or between courses of such treatment (67).

More recently, BMS-275183 was used together with cetuximab (anti-epidermal growth factor receptor monoclonal antibody) in combination therapy. The therapeutic enhancements were more than 1 LCK above the antitumor effect achieved by either single agent applied optimally (68).

Overall, BMS-275183 showed good oral efficacy in five tumor models, as well as a therapeutic advantage in combination therapy, good oral bioavailability and an impressive ability to overcome MDR (R/S ratio = 3.9). BMS-275183 was therefore selected for clinical evaluation as an orally active taxane (64-68).

Other second-generation paclitaxel derivatives

1. BMS-184476

BMS-184476 **(21)** is a C-7 ether-bearing paclitaxel analogue undergoing clinical development by Bristol-Myers Squibb. Studies on the *in vitro* and *in vivo* profile of the compound revealed a tubulin ratio (concentration of test compound giving a change of 0.01 OD/h expressed as a ratio to the concentration of 1) of 1.1 (*vs*. 1.0 for paclitaxel), an IC_{50} for inhibition of HCT 116 cell proliferation of 2.1 nM (*vs*. 1.5-3.5 nM for paclitaxel), a resistance ratio (R/S; IC_{50} for MDR HCT 116 cells/IC_{50} for sensitive HCT 116 cells) of 10.5 (*vs*. 100-200 for paclitaxel) and an LCK value (LCK of 1 considered active) of 1.0 for s.c. M-109 tumors at a dose of 13 or 24 mg/kg/day s.c. x 5 beginning 4 days postimplantation (*vs*. 0.9 for paclitaxel at the MTD) (54, 55).

In the first phase I trial, 34 patients were treated with 78 cycles of BMS-184476 via 1-h infusion every 3 weeks without premedication (contrasting with paclitaxel) at doses ranging from 20 to 80 mg/m^2. The observed DLTs included severe neutropenia with fever, severe diarrhea and/or severe mucositis. The pharmacokinetics of BMS-184476 were linear over the dose range studied. The MTD recommended for phase II studies was 60 mg/m^2 as a 1-h i.v. infusion every 3 weeks (56).

In a subsequent phase I trial, 53 adult patients with solid tumors were treated with BMS-184476 weekly without premedication for 3 consecutive weeks every 28 days, followed by a schedule of weekly administration for 2 consecutive weeks every 21 days. Doses of 7, 14, 28, 40, 50 and 60 mg/m^2/week were investigated. The DLT was neutropenia and the MTD was determined to be 50 mg/m^2/week. Antitumor activity was observed in breast cancer and non-small cell lung cancer patients. Partial responses were confirmed in 22% of patients. Unchanged compound was the main species found in plasma, with < 5% of the observed species consisting of metabolites. The pharmacokinetics of BMS-184476

appeared to be linear at all dose levels studied. Based on these findings, the recommended dose and schedule for further study of BMS-184476 was 50 mg/m^2 on days 1 and 8 every 21 days (57).

2. MAC-321

MAC-321 **(24)** is a novel C-7- and C-3'-modified docetaxel analogue under clinical development by Wyeth. The drug is capable of enhancing the rate of tubulin polymerization *in vitro* and causing the bundling of microtubules in cells. MAC-321 inhibited proliferation of a panel of 14 tumor cell lines with minimal variation in potency (IC_{50} = 2.2 ± 1.4 nM). These cell lines included: 1) A549, HCT 116, NCI-H838, KB-3-1 and 1A9 (A2780 subclone), all of which are sensitive to paclitaxel; 2) MX-1W, NCI-H1299, DLD-1 and HCT-15, all of which are resistant to paclitaxel; and 3) COLO 205, LOX, PANC-1, AsPC-1 and Capan-1, which have an unknown resistance profile. Unlike paclitaxel or docetaxel, the inhibitory potency of MAC-321 did not decrease in cells expressing low to moderate levels of P-gp. Even with KB-V1 cells which highly overexpress P-gp, resistance to MAC-321 was 80-fold compared to the 1,400- and 670-fold resistance detected with paclitaxel and docetaxel, respectively (69).

Against paclitaxel-sensitive Lox melanoma tumors, MAC-321 administered at a dose of 70 mg/kg i.v. achieved 80% tumor inhibition 3 weeks after initial dosing, with a concurrent 9% decrease in body weight. Paclitaxel also performed well in this tumor model when dosed at 60 mg/kg on days 1, 5 and 9. A 95% inhibition of tumor growth was achieved up to day 35, and about 60% of the animals did not have any detectable tumors at this time. In a subsequent efficacy study using the Lox melanoma model, MAC-321 was given orally at doses ranging from 10 to 200 mg/kg. The minimum effective dose of MAC-321 was 20 mg/kg, and a dose of 200 mg/kg was needed to completely inhibit tumor growth. Based on these results, MAC-321 was further evaluated in KB-3-1 and A375SM tumor models, both of which are sensitive to paclitaxel and docetaxel. Upon treatment of tumor-bearing animals with a single dose of MAC-321 at 70 mg/kg i.v., minimal (KB-3-1) or no (A375SM) tumor growth was observed 30 days after dosing. In the same experiment, i.v. paclitaxel (administered as a single dose of 60 mg/kg) demonstrated over 90% tumor inhibition by day 15. However, tumor growth after day 15 was observed in paclitaxel-treated animals in both tumor models. In contrast, no tumor growth was detected in animals treated with i.v. MAC-321. More importantly, MAC-321 also demonstrated pronounced oral efficacy in both models (69).

The antitumor effect of MAC-321 administered by both the i.v. and oral routes was explored in the DLD-1 model. Over 95% tumor inhibition was achieved with i.v. administration of MAC-321 at a dose of 60 mg/kg. The oral activity of MAC-321 was also investigated in the DLD-1 model in a subsequent experiment, and the minimum effective oral dose was determined to be 100 mg/kg. However, tumor regrowth after day 7 was observed in this case. When evaluated against MDR-positive KB-8-5 cervical carcinoma, a single dose of MAC-321 of 70 mg/kg either i.v. or orally significantly inhibited tumor growth. However, tumor regrowth occurred after oral treatment. Similar results were found when animals bearing MX-1W human breast carcinoma were treated with MAC-321. Finally, the antitumor effect of MAC-321 was evaluated in the HCT-15 tumor model (a colon carcinoma inherently resistant to both paclitaxel and docetaxel). In this model, multiple i.v. doses of MAC-321 (20 mg/kg) were needed to achieve > 90% tumor inhibition, and this dose level was associated with a concurrent 7% loss in body weight (69).

In summary, MAC-321 is a novel taxane that is capable of overcoming P-gp-mediated drug resistance both *in vitro* and *in vivo*. This drug can be used in a non-Cremophor EL-containing vehicle via both the i.v. and oral routes. On the basis of these advantages, this compound is being evaluated in phase II clinical trials for the treatment of cancer in humans (69).

3. IDN-5109 (SB-T-101131)

IDN-5109 (SB-T-101131 **[26]**) is a novel 1,14-carbonate taxoid under clinical development by Bayer. The *in vitro* cytotoxicity of IDN-5019 was determined against ovarian A121, non-small cell lung A549, colon HT-29 and breast MCF7 cancer cell lines, giving an average IC_{50} value of 1.1 nM. Unlike paclitaxel and docetaxel, IDN-5019 maintained good inhibitory potency against the paclitaxel-resistant MCF7-R breast cancer and CEM VBL-R leukemia cell lines, with IC_{50} values of 35 and 50 nM, respectively (74, 76).

When tested against androgen-independent DU 145 prostate carcinoma tumor xenografts, i.v. IDN-5109 (45 and 90 mg/kg) displayed a superior and more persistent antitumor effect than i.v. paclitaxel (75). Furthermore, oral antitumor activity against P-gp-positive tumors such as human colon SW620 carcinoma was demonstrated for IDN-5109 at high doses (320-720 mg/kg) (76).

4. DJ-927

DJ-927 **(27)** is a 9β-dihydrobaccatin-9,10-acetal-bearing taxane discovered at the former Daiichi Pharmaceutical (now Daiichi Sankyo). The *in vitro* IC_{50} values for DJ-927 in murine P388 leukemia cell lines, human PC-6 and PC-12 lung cancer cell lines and the resistant PC-6/VCR29-9 and PC-6/VP1-1 cancer cell lines were 0.67, 0.38, 0.62, 2.58 and 21.3 nM, respectively. For comparison, the IC_{50} values for paclitaxel against the same cell lines were 2.93, 1.27, 539, 455 and 1000 nM, respectively. Thus, pronounced enhancement of potency against PC-12 and paclitaxel-resistant PC-6 cell lines was observed for DJ-927 relative to paclitaxel, and the compound was tested further against a wide variety of tumor cell lines, including lung, breast, stomach, pancreatic, colon, ovarian, prostate and brain cancers, as well as leukemia. The average IC_{50} values detected for DJ-927 were 0.55 nM for all cell lines, 0.47 nM for paclitaxel-sensitive cell lines and 0.78 nM for cells expressing P-gp (80, 81).

When evaluated against B16 melanoma *in vivo*, DJ-927 demonstrated potent antitumor effects after both i.v. and oral administration. The effective dose ranges were 7.9-11.0 mg/kg p.o. and 5.3-11.9 mg/kg i.v., whereas no oral efficacy was achieved by docetaxel even at the high dose of 600 mg/kg. In subsequent *in vivo* efficacy studies, DJ-927 was administered orally to mice bearing P-gp-positive DLD-1 colon cancer and DU4475 breast cancer xenografts. In contrast to the outcomes observed with i.v. paclitaxel or docetaxel, significant tumor growth inhibition was observed in DJ-927-treated mice. Furthermore, when tested in an M5076 liver metastasis model (also P-gp-positive), DJ-927 significantly prolonged the lives of tumor-bearing mice when administered via both the i.v. and oral routes (80).

The absorption, distribution, metabolism and excretion (ADME) profile of DJ-927 was investigated in mice, dogs and monkeys after single oral doses. More than 80% of the dose administered to mice was excreted within 48 h, whereas 80% of the dose was excreted by dogs and monkeys in 168 h. Urinary excretion was < 7%. DJ-927 was absorbed rapidly in all three species and widely and extensively distributed to tissues, except the brain. This novel taxane is currently undergoing phase I clinical testing in the U.S. (81).

5. Orally active 10-C-morpholinoethyl docetaxel analogue **28**

When tested against human small cell lung PC-6, human non-small cell lung PC-12 and the P-gp-expressing vincristine-resistant PC-6/CRR29-9 cell lines, compound **28** gave IC_{50} values of 0.27, 0.76 and 9.0 nM, respectively. Mice bearing B16/BL6 melanoma implanted s.c. were used for *in vivo* comparison of the antitumor activity of **28** and docetaxel. Compound **28** showed potent antitumor effects over a wide range of doses following both i.v. and oral administration. Furthermore, although **28** at a dose of 75 mg/kg i.v. was lethal to the animals, oral administration of the same dose to the tumor-bearing mice exhibited potent antitumor effects, with an IR ([1-TWt/Twc] x 100, where TWt is the mean tumor weight of the treated group and TWc is the mean tumor weight of the control group) value of 98.3% and a concurrent body weight loss of only 5.0%. It is worth noting that compound **28** administered i.v. at a dose of 22.2 mg/kg produced impressive antitumor effects, with an IR value of 93.7% and a concurrent body weight loss of 5.7%. In contrast to these findings, oral docetaxel at a dose of 600 mg/kg yielded an IR value of only 6.2%. As expected, docetaxel at 100 mg/kg i.v. achieved very impressive antitumor effects, with an IR value of 95.1% (83-85).

Design and SAR studies for bisindole *Catharanthus* alkaloids

For more than 4 decades, vinblastine **(8)** and vincristine **(9)** have been recognized as clinically important antitumor agents because of their strong antineoplastic activity against a wide spectrum of human tumors (96). These two alkaloids, although structurally almost identical, differ markedly in the type of tumors that they affect and in their toxic properties. In particular, vinblastine is used in the treatment of Hodgkin's disease, while vincristine, which is more widely used in general, is considered the drug of choice in treating acute childhood leukemia (97).

Vinblastine and vincristine are highly potent drugs that also have serious side effects, especially on the neurological system (98). Therefore, it was desirable to identify structural modifications that would yield more effective and less toxic analogues (99). This long-standing interest has led to worldwide research programs to investigate new structurally modified congeners of these alkaloids. Research has mainly focused on the partial or total synthesis of vinblastine analogues from the two parts of the dimeric structure, the Iboga and Aspidosperma portions, by carbon skeleton modification and functional group transformation. To date, these efforts have led to more than a dozen candidates in clinical evaluation. Optimization and SAR development of the vinblastine structure will continue to aid in the discovery of new generations of effective antitumor drugs.

SAR studies on the velbanamine portion (upper moiety): discovery of vinorelbine and vinflunine

Although the upper moiety of vinblastine has been relatively less studied due to its lack of functionalities, it is considered to be a very important region in terms of the potency and novelty of analogues. Modification of the upper velbanamine moiety via the rearrangement of catharanthine is less easily accessible and would require many steps. However, several SAR observations can be deduced from the biological evaluation of various analogues that have been prepared (100).

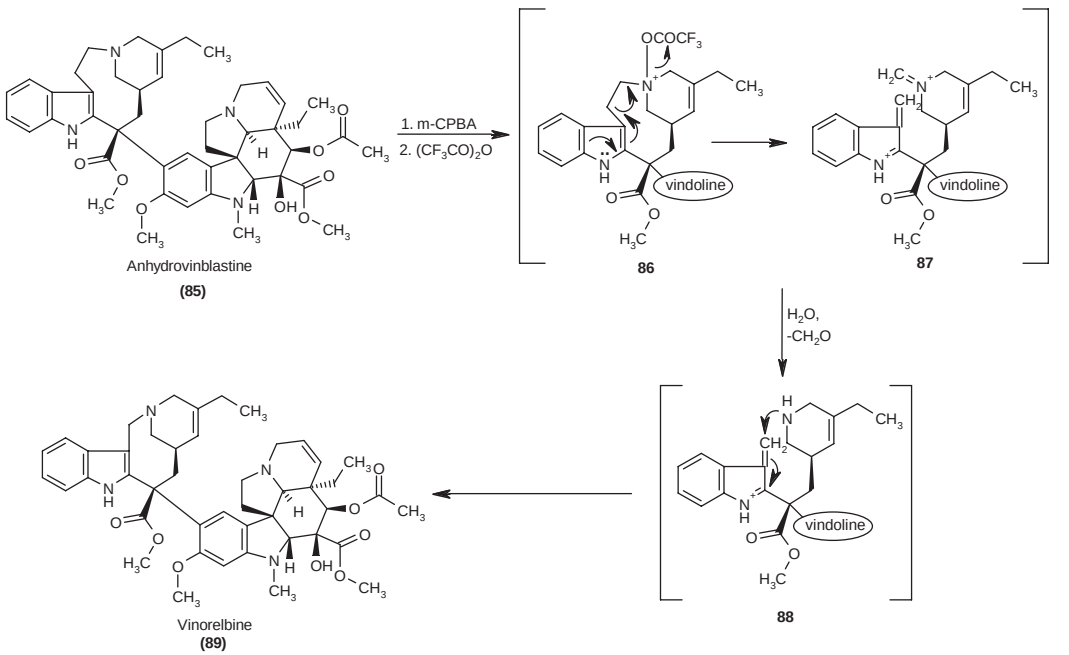
The stereochemical configurations at C-16' and C-14' in the velbanamine portion are critical. Inversion of the configurations leads to loss of activity. The C-16' carbomethoxy group of the velbanamine portion is also important since the decarboxylated dimer is inactive.

Structural variation at C-15'-C-20' of the velbanamine portion is well tolerated. Leurosidine (the C-20' epimer **[10]**), leurosine (the epoxide **[11]**), the C-20' deoxy derivative, the C-15'-C-20' dehydro derivative and the C-20' desethyl derivative all exhibit different inhibitory activities for microtubule assembly (101).

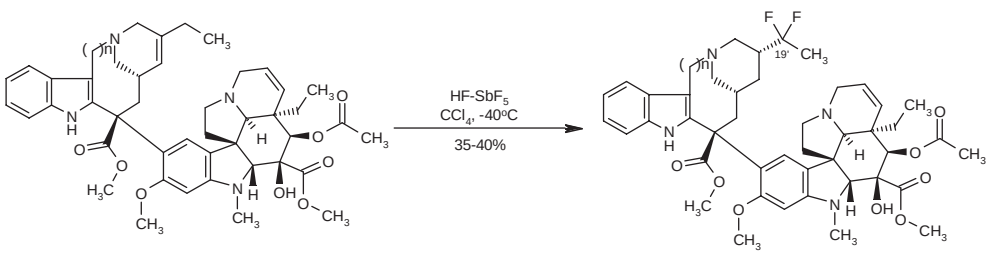
Several N4'-oxides have been prepared by oxidation of the parent compounds with peroxide. They have shown significantly reduced antitumor activity, as well as reduced tubulin binding affinity. Other skeletal modifications, such as removal of the vinblastine D'-ring, have been reported. Vinamidine (also known as catharinine **[13]**), an example with D'-ring cleavage, showed a nearly complete loss of biological activity.

One of the most successful examples of upper region-modified vinblastine analogues is vinorelbine (navelbine **[89]**), which retains excellent activity but is less neurotox-

Scheme 3: Potiers synthesis of vinorelbine via a second modified Polonovski reaction



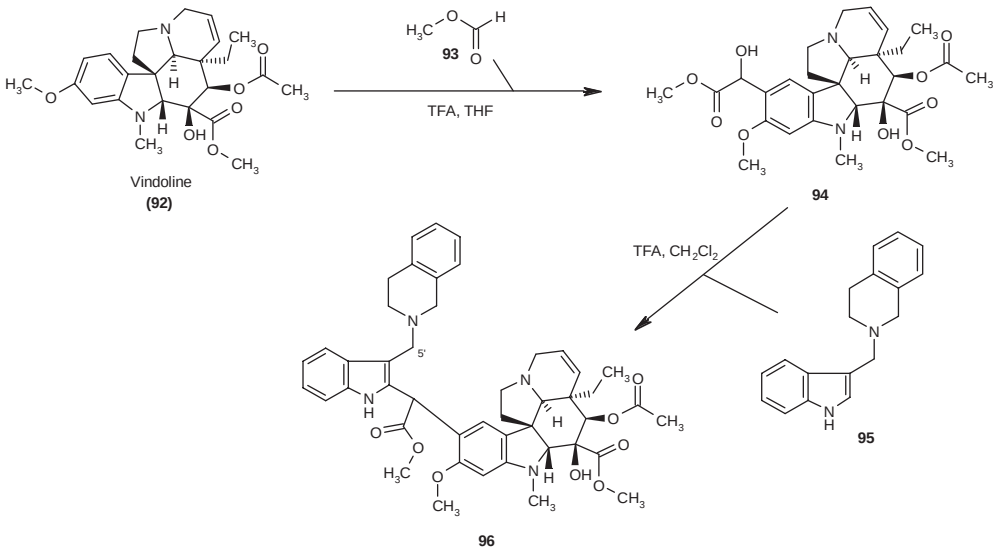
Scheme 4: Synthesis of vinflunine and difluorodeoxyvinblastine via superacid chemistry



ic than vincristine. It was prepared by a second Potier-Polonovski reaction with anhydrovinblastine **(85)**. As shown in Scheme 3, the resultant bisiminium species was hydrolyzed and recyclized to provide skeleton-modified vinorelbine (102-104). The antitumor activity of vinorelbine has been evaluated in numerous murine tumor models. Results have shown remarkable activity against leukemia, although it was inactive in carcinoma and fibrosarcoma models. Vinorelbine is currently available worldwide for the treatment of non-small cell lung and breast cancer. It is orally active, and its use will almost certainly be extended to the treatment of other types of cancer.

Superacid chemistry has been applied to prepare vinflunine (F-12158 **[90]**), the difluoro derivative of vinorelbine (see Scheme 4). This compound has improved *in vivo* antitumor activity as compared to vinorelbine against B16 melanoma and a panel of human tumor xenografts. It is currently undergoing phase I clinical evaluation (105). Similarly, a new family of anhydrovinblastine and vinorelbine analogues was prepared using superacid chemistry. Halogen atoms, ketone and hydroxy groups were introduced into the upper velbanamine portion (106). It was discovered that fluorination at the C-19' position of vinorelbine dramatically increases the *in vivo* activity, while hydroxylation at the same position results in a total loss of activity. In the anhydrovinblastine series, monohalogenation of the C-19' position does not affect activity, but dihalogenation significantly decreases activity. The

Scheme 5: A new strategy to synthesize upper region-modified vinblastine analogues

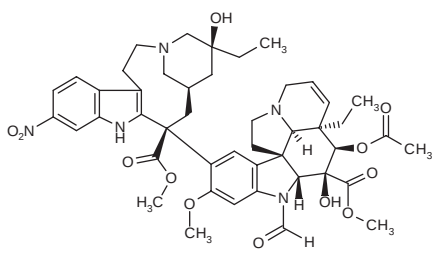


same modifications greatly decrease the *in vivo* activity in the vinorelbine series. In addition to the above findings, deacetylation at the C-17 position of dihydrovinorelbine and vinflunine resulted in increases in both *in vitro* cytotoxicity and *in vivo* activity.

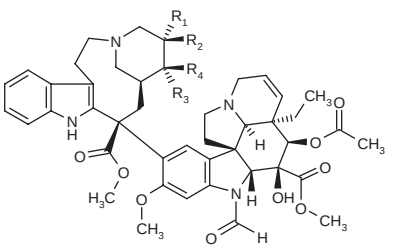
Another strategy for synthesizing new upper region-modified vinblastine analogues has been established (Scheme 5) (107). The preliminary SAR results suggest that the integrity of the velbanamine skeleton in the bisindole alkaloids is important for maintaining their potency. Only one of the tetrahydroisoquinoline derivatives **(96)** exerted marginal activity in the tubulin polymerization inhibition test. Other derivatives lacking the C-5'-C-6' bond in the C'-ring were found to be inactive.

A series of nitration analogues of vincristine reported recently were tested against P388 leukemia *in vivo*. The results showed that the most potent compound **(97)** can increase the life span of mice with P388 leukemia to 217% of that of controls at a dose of 8.0 mg/kg/day (108).

Over 60 congeners of vinblastine have been synthesized, primarily with modifications on the piperidine ring of the carbomethoxycleavamine moiety. These congeners were evaluated for their cytotoxicity against murine L1210 leukemia and RCC-2 rat colon cancer cells, as well as for their ability to inhibit the polymerization of microtubular protein. Two C-15'-C-20' ring *cis*-fused deoxyvinblastine congeners demonstrated extraordinarily high cytotoxicity against L1210. The cytotoxicity of the C-20' S 6-membered ring compound **(98)** was 500 times more potent and the corresponding C-20' R-fused ring compound **(99)** was 1,000 times more potent than vinblastine against L1210 leukemia cells (109).



97

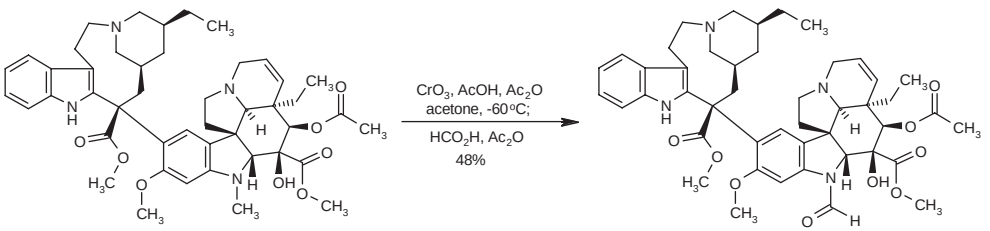


98, $R_2 + R_4 = -(CH_2)_4-$, $R_1 = R_3 = H$
99, $R_1 + R_3 = -(CH_2)_4-$, $R_2 = R_4 = H$

SAR studies on the vindoline portion (bottom moiety)

Most of the SAR studies involve the vindoline portion of bisindole alkaloids. This is due to the fact that the presence of various functionalities, such as the C-16 and C-17 positions, offers good opportunities for preparing new analogues. Many functional groups at C-16 have been found to be equivalent to the ester "pharmacophore"

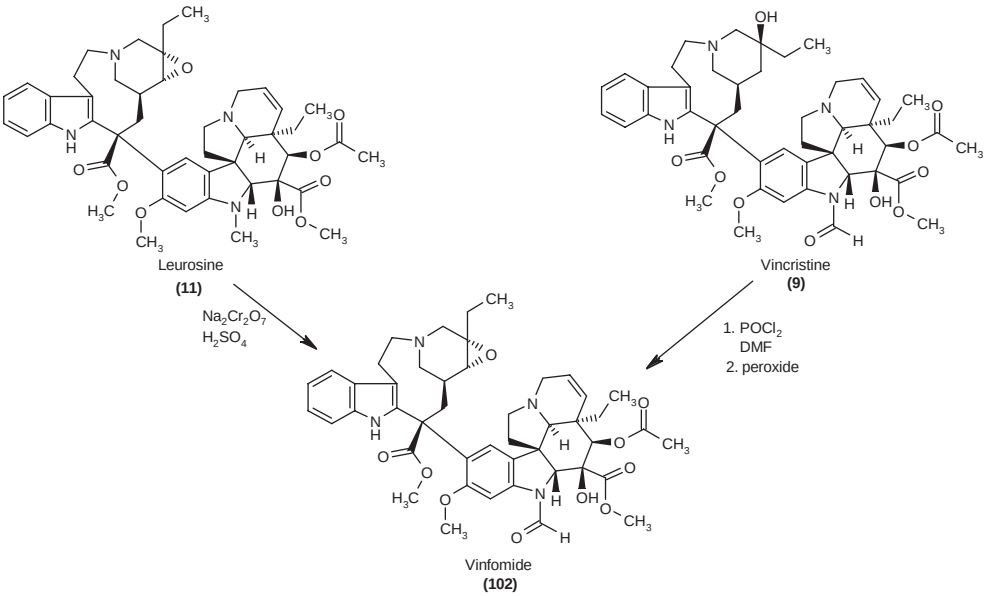
Scheme 6: Synthesis of vinepidine



20'-*epi*-Deoxyvinblastine
(100)

Vinepidine
(101)

Scheme 7: Synthesis of vinformide



at this position. These findings have led to the discovery of several new generations of analogues, such as vindesine and vinzolidine.

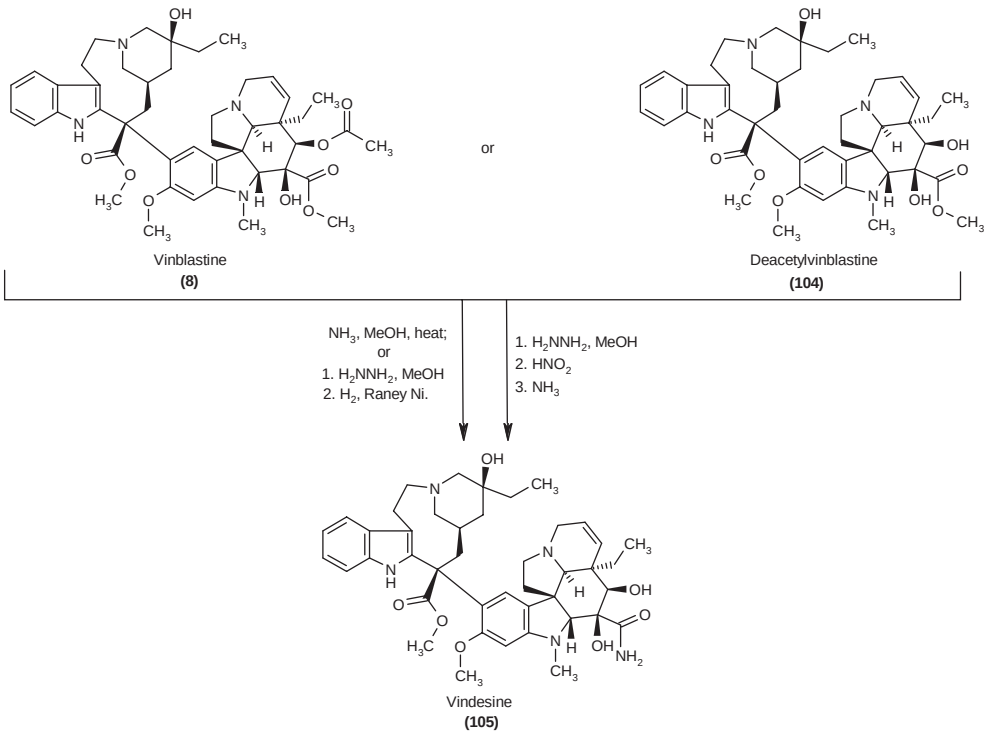
Vinepidine (LY-119863 **[101]**) is a 20'-deoxy epimer of vincristine (110). As shown in Scheme 6, it can be prepared from 20'-*epi*-deoxyvinblastine by oxidation of the vindoline portion's methyl group. In terms of antitumor activity, vinepidine was more effective than vincristine against lymphosarcoma and leukemia. Although its biochemical pharmacology, antitumor efficacy and potency led to a phase I clinical study, neuromuscular toxicity prevented its further clinical development (100, 111).

Vinformide **(102)**, also known as *N*-formylleurosine, is an *N*-formyl analogue of leurosine. It was prepared (Scheme 7) from leurosine by oxidation of the vindoline portion methyl group with various oxidants, including chromic acid (112). Another synthesis started from vincristine reacted with Vilsmeier reagent to afford anhydrovincristine, which was later epoxidized to yield vinformide (113). Vinformide was found to be 20 (P388 cells) to 1,000 (K-562 cells) times less cytotoxic than vincristine and is useful in the treatment of lymphoma, leukemia and Hodgkin's disease. However, despite its promising antineoplastic potency, vinformide also exerts acute cardiotoxic effects (114).

Other N-1 derivatives **(103a-c)** have been reported. Many of them have demonstrated antitumor activity, as well as an improved therapeutic index compared with vinblastine. This finding indicates that vindoline's indole methyl group is a useful position to functionalize and provide potentially more potent vinblastine derivatives (115).

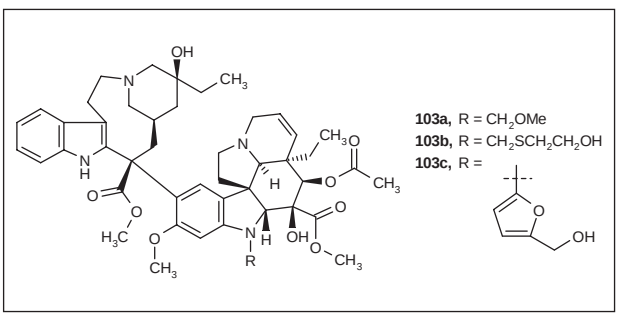
Vindesine (LY-99094 **[105]**) is a chemically modified vinblastine analogue that differs slightly from vinblastine by having an amide group instead of an ester group at C-16. Such minor differences are responsible for the profound alteration in the antitumor spectrum, potency and toxicity of these compounds. In particular, vindesine possesses an antitumor spectrum against rodent tumor sys-

Scheme 8: Synthesis of vindesine



tems that resembles vincristine rather than its parent vinblastine, while its neurotoxic potential appears to be less than that of vincristine. Vindesine is prepared from vinblastine or deacetylvinblastine **(104)** by various methods, as shown in Scheme 8 (116).

The selection of vindesine for clinical evaluation provided an opportunity to explore the consequences of minor structural changes within the "bottom" vindoline portion. Clinical trial data indicated that vindesine is an active oncolytic agent that appears to be less neurotoxic than vincristine. Further studies also indicated that vindesine is active in vincristine-resistant childhood leukemia. When used in combination with cisplatin, vindesine showed efficacy against non-small cell lung cancer. It is currently available for the treatment of leukemia in several countries.

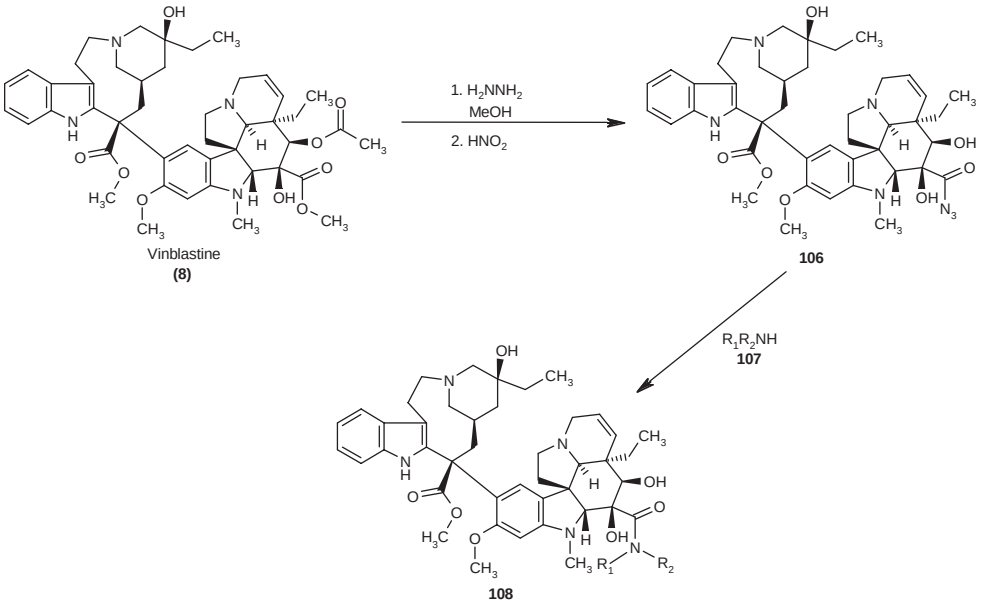


An extensive series of *N*-substituted vindesine analogues **(108)** (Scheme 9) have been prepared from the reaction of deacetylvinblastine acid azide **(106)** with the appropriate amines. These *N*-alkylvindesines have reduced activity. In terms of overall antitumor activity, vindesine emerges as the congener with optimum qualities (117).

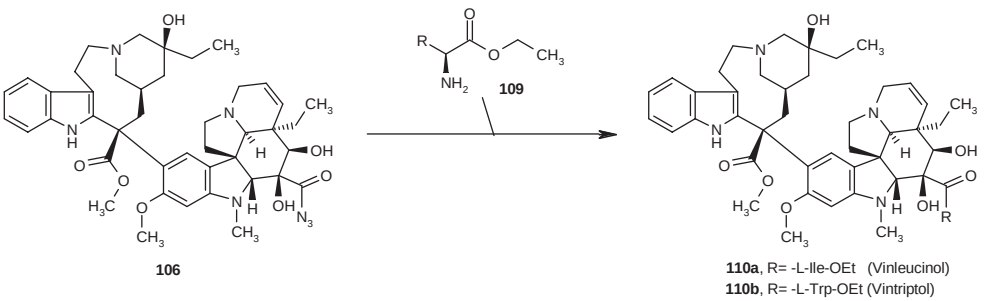
Similarly, 21 vinblastine C-16 carboxyl amino acid derivatives **(110)** (Scheme 10) were synthesized by linking amino acid carboxylic esters to the vinblastine C-16 carboxyl moiety through an amide linkage. The influence of the nature of the amino acid, ester alkyl chain lengths, the stereoisomerism of the amino acid and the re-acetylation of the hydroxyl group at the C-17 position of the vindoline moiety was studied. Among the 21 congeners, vinblastine-L-Trp-OEt (vintriptol **[110b]**) and vinblastine-L-Ile-OEt (vinleucinol **[110a]**) stood out as the derivatives with the best antitumor activities. Further studies also showed that the presence of a tryptophan at the C-16 carboxyl moiety diminished toxicity. Therefore, vinblastine-L-Trp-OEt **(110b)**, also known as vintriptol, was selected for phase I and II clinical trials (118, 119).

A series of new amino phosphonic acid derivatives of vinblastine (Scheme 11) have been synthesized and tested *in vitro* and *in vivo* for antitumor activity. All of these compounds were capable of inhibiting tubulin polymerization *in vitro*. The antitumor activity strongly depended on the stereochemistry of the phosphonates, with the most active com-

Scheme 9: Preparation of vindesine analogues (*N*-alkylvindesines)



Scheme 10: Synthesis of vinblastin-16-oyl amino acid derivatives



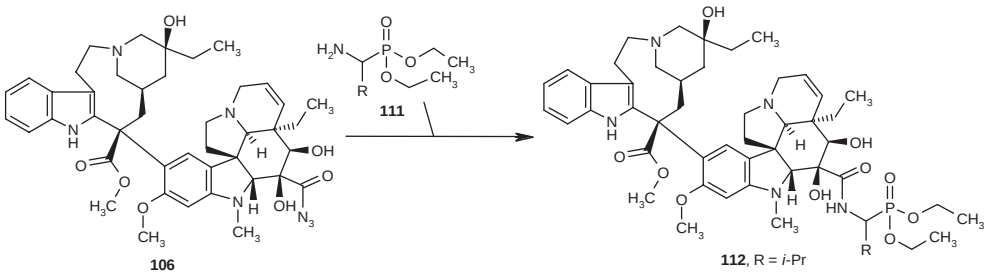
pounds having the (*S*)-configuration. One of them, a compound with an isopropyl group (R = i-Pr) **(112)**, was progressed to phase I clinical trials due to its marked activity against cancer cell lines both *in vitro* and *in vivo* (120).

Cephalosporin substituted at the C-3' position with the potent oncolytic agent desacetylvinblastine hydrazide was synthesized as a potential prodrug for the treatment of solid tumors. The design of this novel prodrug was based on the knowledge that hydrolysis of cephalosporin's β-lactam bond may result in the expulsion of the C-3' substituent. As shown in Scheme 12, treatment of these candidate prodrugs **(113-115)** with the P99 β-lactamase enzyme efficiently catalyzed their conversion to the free drug form (121, 122).

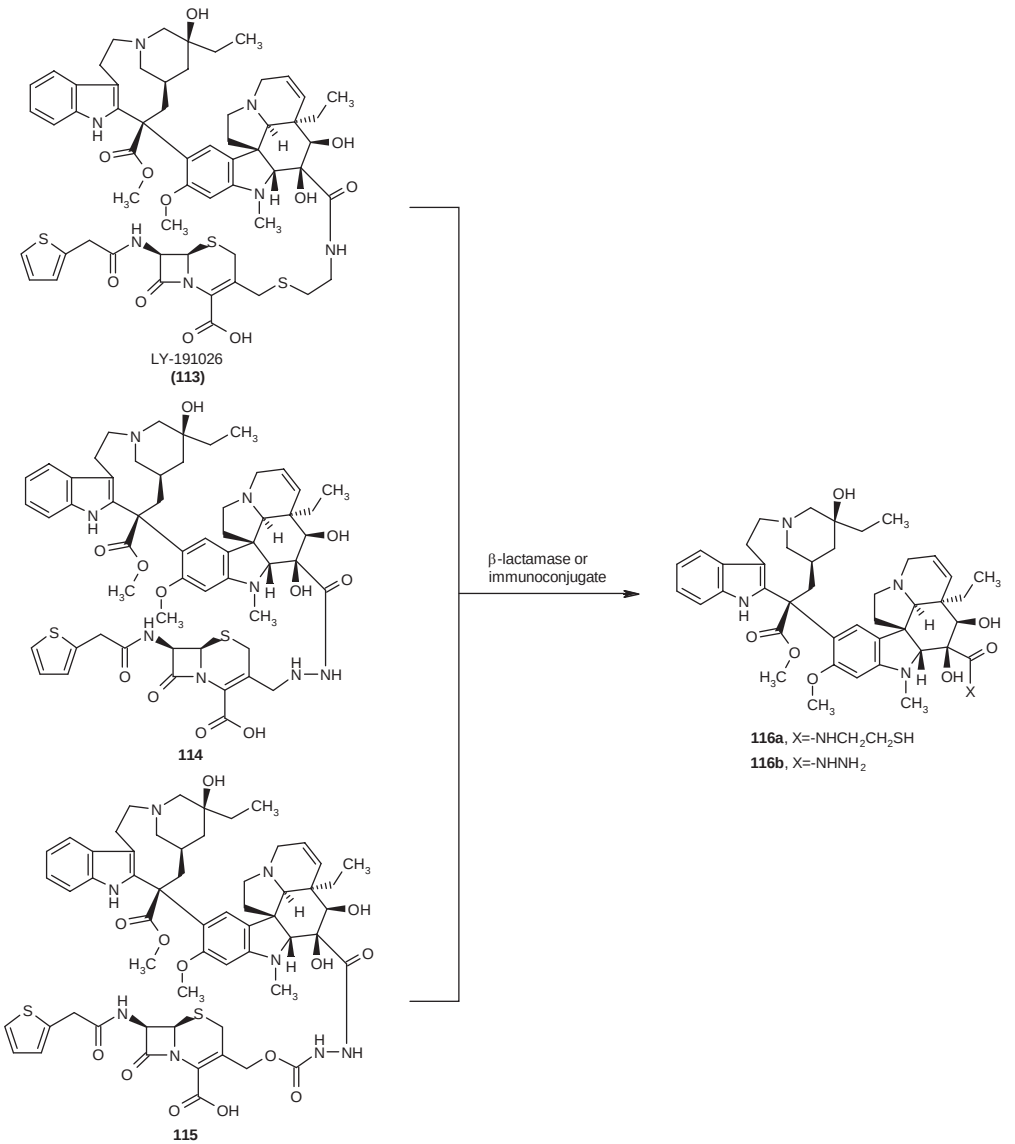
Napavin **(118)**, a photoreactive compound, was synthesized from vinblastine (Scheme 13). In contrast to vinblastine, which binds noncovalently to the intracellular protein tubulin, napavin was designed to form a covalent bond with target proteins upon irradiation in order to prevent its elimination and thus increase the half-life of its action. It was found that napavin, used as a new photoreactive cytostatic substance, can overcome the multidrug resistance of tumor cells (123).

Different from the vast majority of C-16 vinblastine analogues, the C-16 spiro-fused derivatives represent a novel class of semisynthetic vinblastine analogues. In particular, the C-16 spiro-fused oxazolidine-1,3-diones **(119a-c)** can be prepared in two ways, as shown in Scheme 14. A number of bisindole oxazolidinedione analogues have been prepared using these methods, and their antimitotic and antitumor efficacies have been assessed. Due to its exceptional activity and excellent absorption when administered orally, vinzolidine (LY-104208 **[119c]**) was tested in further clinical studies (124, 125).

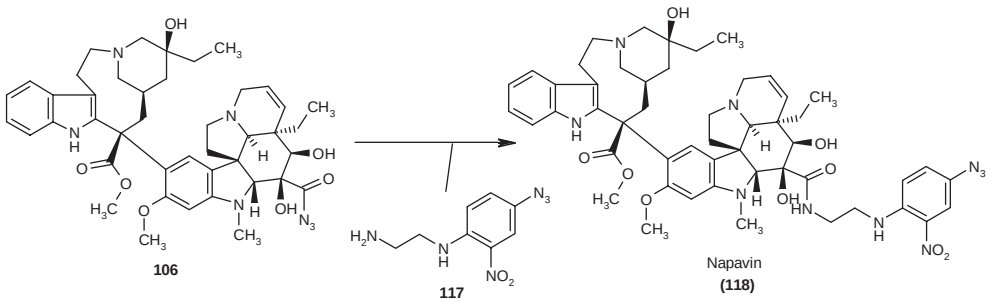
Scheme 11: Synthesis of α-amino phosphonic acid derivatives of vinblastine



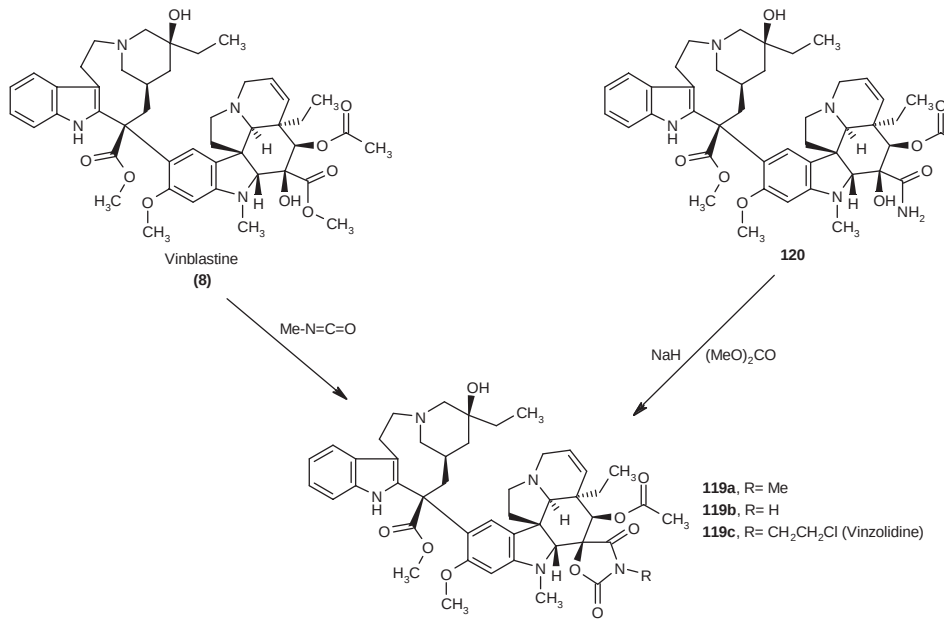
Scheme 12: Synthesis of various cephalosporin-vinblastine prodrugs



Scheme 13: Synthesis of napavin

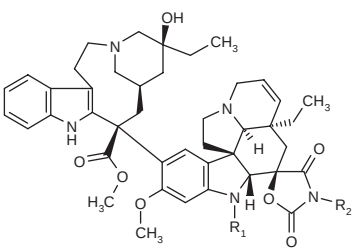


Scheme 14: Synthesis of vinblastine analogues



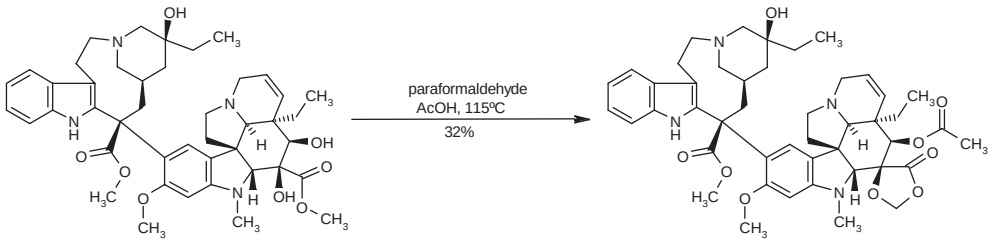
A spirolactone **(121)** was formed during the reaction of paraformaldehyde with deacetylvinblastine **(104)** under acidic conditions (Scheme 15), and its chemotherapeutic activity was assessed against experimental P338 leukemia. The data showed that this compound had much better activity than vinblastine (126).

A new series of semisynthetic C-16 spiro-oxazolidine-1,3-diones **(122a-c)** prepared from 17-deacetoxy-vinblastine using a standard method, and their activities were examined in a number of tests. Of these vinzolidine analogues, compound **122b** showed *in vivo* effects against P388 leukemia at a dose of 20 mg/kg, with lower toxicity than vinblastine and different pharmacological activity The IC_{50} for cytotoxicity was 0.31 μM and the IC_{50} for the antitubulin effect was 136 nM. The spiro-oxazolidine ring and the substitution of a formyl group for a methyl group were responsible for the unique pharmacological effects observed. The studies also showed that these compounds displayed their cytotoxic activity at significantly higher concentrations than the parent compounds, although their antimicrotubular activities were similar *in vitro* (127).



122a, $R_1 = CH_3$, $R_2 = CH_2CH_2Cl$
122b, $R_1 = CHO$, $R_2 = CH_2CH_2Cl$
122c, $R_1 = CH_3$, $R_2 = CH_2CH{=}CH_2$

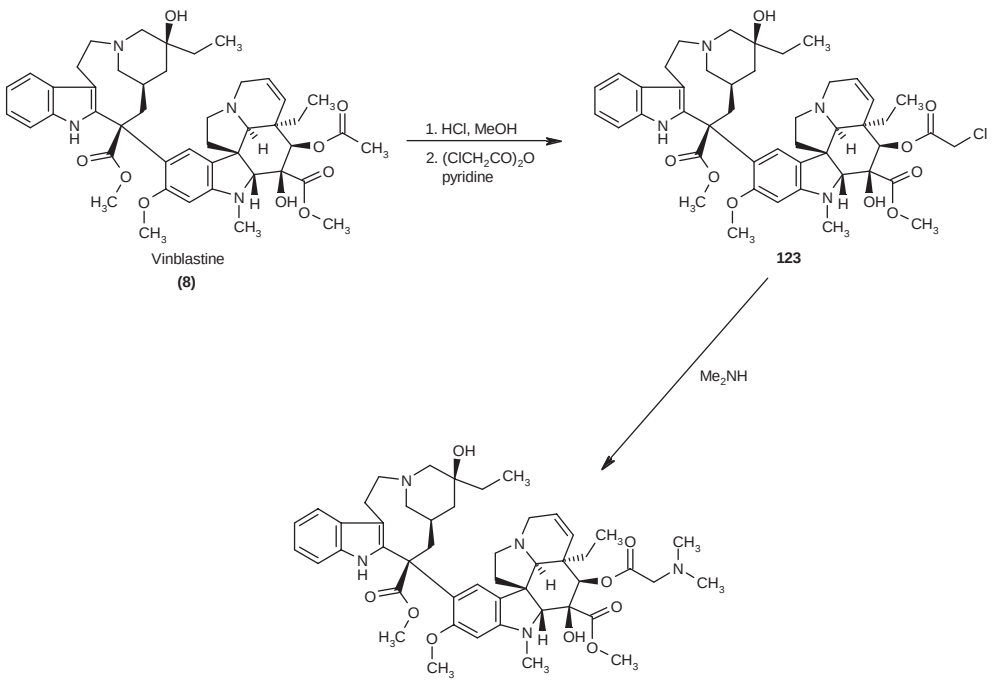
Scheme 15: Synthesis of vinzolidine analogues



Deacetylvinblastine
(104)

121

Scheme 16: Synthesis of vinglycinate



Vinblastine
(8)

123

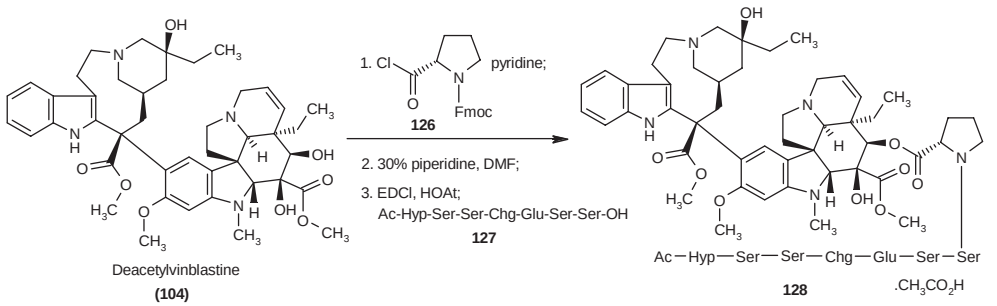
Vinglycinate
(124)

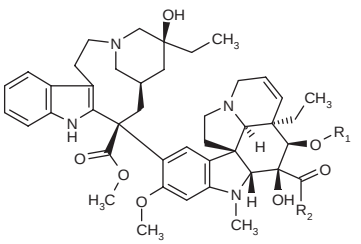
Vinglycinate (LY-49040 **[124]**) is a glycinate prodrug derived from the C-17 hydroxy group of vinblastine. It was prepared from vinblastine, as shown in Scheme 16. Vinglycinate demonstrated measurable activity in several leukemia models and was orally absorbed. Its toxicity and experimental antitumor spectrum of activity were found to be very similar to those of vinblastine. As a result, vinglycinate was progressed to clinical studies. However, further clinical development was stopped because it showed no improvement over vinblastine in terms of efficacy or toxicity (100, 128).

Using a similar method of preparation as for vinglycinate, a series of 17-(α-aminoacyl) derivatives **(116b, 125a-c)** were prepared. The *in vitro* antitumor activities of these compounds were examined, and it was shown that the larger size of the acyl substitutent at C-17 significantly reduced antitumor activity. Several analogues derived from the C-17 hydroxyl group were prepared and their activities tested. The most potent, compound **116b**, has an IC_{50} value of < 0.0003 μg/ml in P3UCLA cells and an IC_{50} value of 0.03 μg/ml in CEM cells (129).

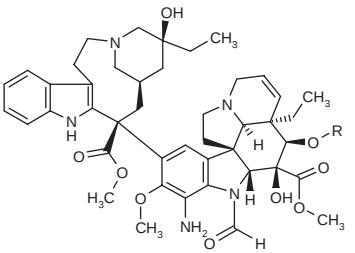
A series of peptidyl conjugates at the C-17 position of vinblastine have been developed for solving the issues of marginal efficacy and dose-limiting systemic toxicity. Compound **128** (Scheme 17) was evaluated as a prodrug targeted to prostate cancer cells. This compound features

Scheme 17: Synthesis of peptidyl prodrugs of vinblastine





116b, R_1 = H, R_2 = $NHNH_2$
125a, R_1 = H, R_2 = OCH_3
125b, R_1 = $COCH_3$, R_2 = OCH_3
125c, R_1 = $CO(CH_2)_2CO_2H$, R_2 = OCH_3



129a, R = Ac
129b, R = H

an octapeptide segment attached by an ester linkage at the C-17 position of vinblastine, and it undergoes rapid cleavage ($t_{1/2}$ = 12 min) by prostate-specific antigen (PSA) between the Gln and Ser residues. In nude mouse xenograft studies, it reduced circulating PSA levels by 99% and tumor weight by 85% at a dose just below its MTD (130).

Several amino derivatives were prepared from the bisindole alkaloids vinblastine and vincristine, as well as from *N*-formylleurosine, by nitration and subsequent reduction. The antitumor activities were tested in 64 human tumor cell lines. Two of these compounds **(129a, 129b)** demonstrated cytotoxic activity against non-small cell lung and breast cancer cell lines in the concentration range tested (0.001-10 μM). Compound **129a** also demonstrated activity against colon cancer and leukemia (108).

Summary and future perspectives

Since the discovery of *Taxus* diterpenoids and bisindole *Catharanthus* alkaloids, tremendous efforts have been made in synthetic and medicinal chemistry to develop and refine new compounds. Both paclitaxel and vinblastine have served as lead structures for discovery chemistry and lead optimization.

With the goal of identifying paclitaxel derivatives retaining *in vivo* efficacy against paclitaxel-resistant tumors, scientists from the pharmaceutical industry and academic institutions synthesized and evaluated various paclitaxel analogues containing modifications at the core and side-chain regions. As a result of this intensive effort, several second-generation paclitaxel derivatives, such as BMS-184476, BMS-188797 and IDN-5109, were selected for clinical evaluation against solid tumors on the basis of their excellent *in vitro* and *in vivo* activity in a number of animal models. The future success of these novel taxanes in clinical trials should provide us with more treatment options for combating various paclitaxel-resistant cancers. In addition, the success of orally active paclitaxel analogues in clinical trials should reduce the cost of chemotherapy by eliminating the need for premedication, as required for paclitaxel treatment.

The discovery of the bisindole alkaloids and their biosynthetic precursors, which occur in the same plant, has culminated in the development of many novel efficient synthetic routes that are applicable for SAR study and process development. These efforts have enriched our knowledge in both synthetic organic chemistry and medicinal chemistry research areas. The original biosynthetic hypothesis and subsequent synthetic studies led to an understanding of the biosynthetic pathway, allowing us to couple both catharanthine and vindoline to access bisindole alkaloids. As a result, many natural and semisynthetic analogues of vinblastine were prepared using

the biosynthetic concepts and newly developed methodologies. In addition, other areas of pharmacokinetics and pharmacology have been extensively explored, and several related disciplines have been integrated. For example, SAR studies have led to an understanding of various pharmacophores present in the molecule. Medicinal chemistry also allows us to probe the relevant biology, especially the mode of action and tubulin-microtubule dynamics. New generations of vinblastine analogues, represented by vinorelbine, vindesine and vinzolidine, as well as vinflunine and many others, have been brought into clinical studies, and several of them have become successful anticancer drugs.

Future progress will focus on the necessity of developing a 3-dimensional structure of the paclitaxel or vinblastine binding sites, which will benefit rational drug design. Such a ligand-receptor model will also allow us to probe the tubulin-microtubule dynamics at the molecular level. Meanwhile, a suitable screening model for clinically useful *Taxus* and bisindole alkaloids needs to be defined. Other research horizons will arise from the development of bioconjugates or hybrids of these alkaloids with other biologically active molecules, as well as from our increasing knowledge of the molecular mechanisms of drug resistance.

In conclusion, the discovery of the *Taxus* and *Catharanthus* family of natural products will continue to provide good opportunities to explore chemical biology, leading to the generation of new clinically useful drugs for the treatment of human cancers.

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