The Role of Microtubules in Cell Biology, Neurobiology, and Oncology

Cancer Drug Discovery and Development

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Edited by

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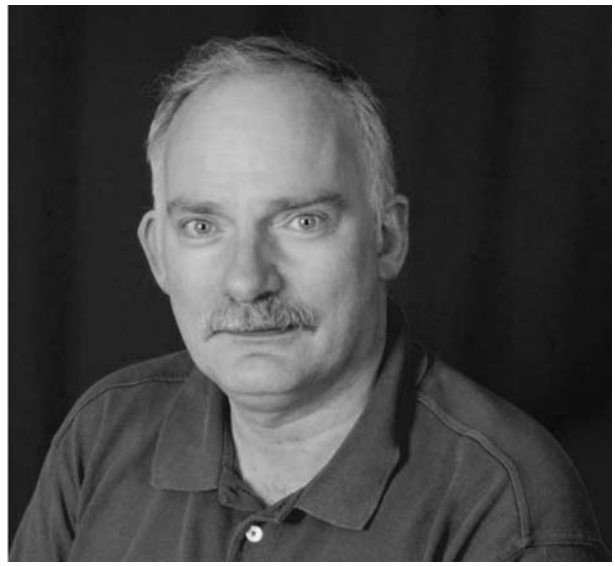

This book is dedicated to the memory of Dr. George A. Orr, a dear friend and colleague. George was a talented and creative scientist who set very high standards for himself and expected the same from his collaborators. He loved to share concepts and ideas, and many of us had the privilege of interacting and collaborating with him. He inspired and encouraged many young scientists and stimulated them to do their best work. About his own accomplishments, he was extremely humble.

George did his undergraduate and PhD studies at Queen's University, Belfast, N. Ireland. His postdoctoral work was done with Dr. Jeremy Knowles at the University of Oxford and then at Harvard University. George came to the Albert Einstein College of Medicine as an assistant professor in the Department of Molecular Pharmacology in 1978 and rose through the ranks to become a full professor in 1989.

George had many scientific interests but he was particularly dedicated to the development and application of new technologies to enhance our insights into the mode of action of drugs. His participation in the field of microtubule pharmacology and proteomics has opened new avenues of research to all of us. He is sorely missed.

PREFACE

I want to thank all who contributed to this first edition for their hard work and professionalism, and especially for their patience. I hope the readers will find this volume as helpful as I have found it.

There is no doubt that the family of proteins we call the tubulins and the microtubules that they form when they aggregate are extremely important in the cell and, as we are increasingly learning, important in diseases that afflict so many. This field of investigation is a testament to how important both basic and clinical sciences are in understanding disease mechanisms and making inroads into therapies. Without the basic science knowledge that has been accumulated, to which the authors of this work have contributed greatly, we would not be in the position we find ourselves of increasingly understanding disease and advancing therapies. As I read the chapters, I was humbled to think of the insights that so many have contributed to this field, and again became aware of how the collaborative effort of so many is needed to understand the complexities of nature. By working together, many have helped to advance this field. Because of their efforts, we find ourselves with the wealth of knowledge contained in this book. This knowledge gives us so much insight even as it challenges us to continue working. Thanks again to all of the wonderful collaborators for their excellence and their patience.

Tito Fojo, MD, PhD

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1 An Overview of Compounds That Interact with Tubulin and Their Effects on Microtubule Assembly

Ernest Hamel

Contents

Summary

Over the last quarter of a century new classes of compounds that interfere with cell division as a result of binding to tubulin αβ-dimers, oligomers, or polymers have been described with seemingly ever-greater frequency. The cytological hallmark of tubulin interactive agents is the accumulation in drug-treated cell cultures of a high proportion of cells that appear to be arrested in mitosis. These cells have condensed chromosomes, no nuclear membrane, and a deformed or absent mitotic spindle. Flow cytometry for DNA content demonstrates large numbers of tetraploid (G2/M) or even octaploid cells.

Key Words: Tubulin; taxoid site; laulimalide site; tubulin alkylation; colchicine site; vincer domain.

1. INTRODUCTION

Antimitotic agents vary widely in molecular structure, even among compounds that appear to interact in the same binding region of tubulin, and represent natural products obtained from a wide variety of organisms, synthetic compounds, and synthetic analogs of the former. Antitubulin compounds range in complexity from the simple sulfhydryl reagent 2,4-dichlorobenzyl thiocyante (MW, 204) to the macrocyclic polyether halichondrins and spongistatins (MWs > 1100). The current interest in drugs that interact with tubulin is a result in large part of the clinical importance of the taxoids paclitaxel and docetaxel as anticancer agents *(1,2)*, but also to the possibility that additional agents might overcome resistance to antimitotic therapy, have reduced toxicity in patients,

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and/or that antitubulin drugs used in combination might have synergistic clinical benefit, as has been repeatedly observed in cell culture studies *(3–17)*. Moreover, drugs that interact with tubulin are useful in the treatment of parasitic diseases of humans and animals *(18)*, and colchicine remains important as a treatment for familial Mediterranean fever and occasionally for other inflammatory conditions *(19)*.

2. COMPOUNDS THAT INDUCE TUBULIN ASSEMBLY BY BINDING AT THE TAXOID OR LAULIMALIDE SITES

Currently, there are at least five well-described modes by which antimitotic agents interact with tubulin. In the first two modes, the compound is believed to bind with high affinity to polymerized tubulin vs the αβ-tubulin heterodimer. There are at least two polymer-based binding sites, to which drugs can bind, and this discussion will be restricted to the lead compounds, all of them natural products, with substantial cytotoxic activity (arbitrarily defined as yielding reported IC50s less than 50 nM). There are now eight such natural products.

The best described of the two binding sites is that to which paclitaxel *(20)* binds *(21)*. Besides paclitaxel, natural products known to bind to the taxoid site are epothilones A and B *(22,23)*, discodermolide *(24,25)*, eleutherobin *(26,27)*, cyclostreptin (also known as FR182877) *(28–31)*, and dictyostatin 1 *(32–34)*. The structures of these compounds are shown in Fig. 1. The first publication describing the assembly promoting properties of paclitaxel appeared in 1979 *(20)*, and it was not until 1995 that a paper describing additional assembly inducing compounds, epothilones A and B, appeared *(22)*.

The assembly promoting properties of laulimalide (structure in Fig. 2) were initially described in 1999 *(35)*. Laulimalide has been shown to bind to a different site than the taxoid site on tubulin polymer *(36)*. The evidence for a different site for laulimalide is its failure to inhibit the binding of taxoids to tubulin polymers, its stoichiometric incorporation with paclitaxel into polymer *(36)*, and its ability to act synergistically with paclitaxel in promoting tubulin assembly *(37)*. A second assembly promoting compound, peloruside A *(38)* (structure shown in Fig. 2), has been found to bind at the same site as laulimalide. Peloruside A does not inhibit taxoid binding to polymer but does inhibit laulimalide binding *(39)*.

These eight natural products lead to the hyperassembly of microtubules of enhanced stability in cell free reaction mixtures. Typically, assembly reactions can occur under conditions where there is normally little or no tubulin polymerization: at reduced temperatures, in the absence of GTP, in the absence of microtubule-associated proteins or another assembly promoting reaction component (such as glycerol or glutamate), and at reduced tubulin concentrations. These properties indicate hypernucleation of assembly, and consistent with this conclusion is the routine observation that the microtubules formed with drug are shorter and more abundant than microtubules formed without drug. Microtubules formed in the presence of drug are also resistant to disassembly induced by cold temperatures, dilution, or Ca^{2+}. In all cases studied thus far, assembly promoting compounds bind to tubulin polymer stoichiometrically (i.e., in an amount equivalent to the tubulin content of the polymer; for e.g., *see* ref. *36*). In cells, assembly promoting drugs cause formation of abnormal spindles in mitotic cells (those with condensed chromosomes), whereas in interphase cells the drugs cause increased numbers of misorganized microtubules that are typically shorter and bundled as compared with the microtubule cytoskeleton of control cells.

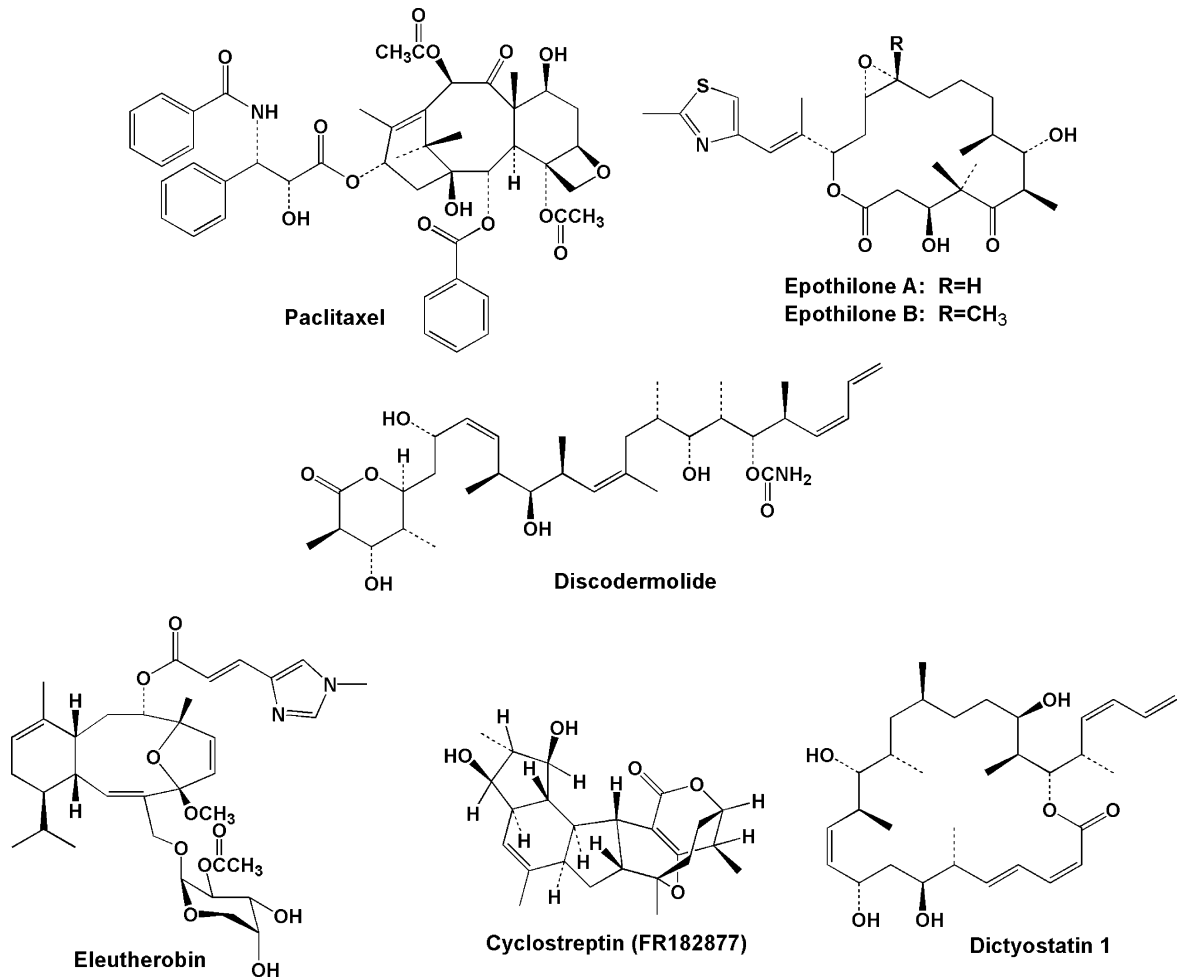


Fig. 1. Structures of paclitaxel, epothilones A and B, discodermolide, eleutherobin, cyclostreptin, and dictyostatin 1. The configurations of the chiral center, shown for dictyostati are as proposed by Paterson et al. *(33,104)*.

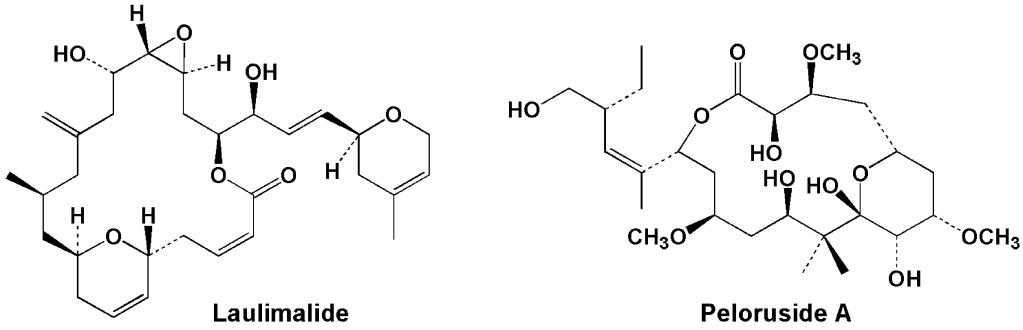


Fig. 2. Structures of laulimalide and peloruside A.

3. COMPOUNDS THAT INHIBIT TUBULIN ASSEMBLY BY ALKYLATING β-TUBULIN OR BY BINDING IN THE COLCHICINE SITE OR THE VINCA DOMAIN

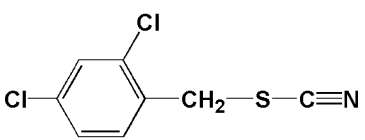
Drugs interacting with tubulin by the other three well-described mechanisms all inhibit microtubule assembly. These agents either alkylate tubulin amino acid residues, bind in the colchicine site, or bind in the vinca domain. As a general rule, in cells treated with compounds that inhibit tubulin assembly, microtubules disappear. At lower drug concentrations, mitotic cells display abnormal spindles, whereas at higher concentrations there is no spindle at all. The microtubule network in interphase cells becomes progressively sparser until it completely vanishes as the drug concentration increases.

Highly specific covalent bond formation with tubulin amino acid residues, generally cysteines, occurs with at least four compounds that arrest cells in mitosis (structures in Fig. 3). Alkylation of Cys-239 of β-tubulin alone causes loss of tubulin's ability to polymerize. The covalent interaction of 2,4-dichlorobenzyl thiocyanate with tubulin occurs at multiple cysteine residues, but Cys-239 of β-tubulin is the most reactive *(40)*. The reaction with Cys-239 eliminates the ability of tubulin to assemble into polymer but has much less effect on the ability of tubulin to bind either colchicine or GTP. The IC_{50} of 2,4-dichlorobenzyl thiocyanate for inhibition of growth of two cell lines was in the 200–500 n*M* range *(41)*.

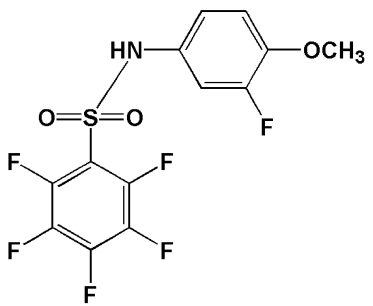
Subsequently, 2-fluoro-1-methoxy-4-pentafluorophenylsulfonamidobenzene (T138067) *(42)* was shown to react exclusively with Cys-239. T138067, which has structural analogy to colchicine site drugs, inhibits the binding of colchicine (structure shown in Fig. 4) to tubulin, and colchicine inhibits the covalent interaction of T138067 with β-tubulin. The IC_{50}s of T138067 with a number of cell lines were in the 10–50 n*M* range.

Originally, 4-*tert*-butyl-[3-(2-chloroethyl)ureido]benzene, an arylchloroethylurea, was also thought to react exclusively with Cys-239, and 4-*tert*-butyl-[3-(2-chloroethyl)ureido]benzene was cytotoxic toward several cell lines in the low micromolar range *(43)*. Newer analogs *(44)* have somewhat improved cytotoxicity and alkylate β-tubulin in cells more rapidly. However, subsequent work has shown that this class of drugs alkylates intracellular tubulin at Glu-198 of β-tubulin *(45)*.

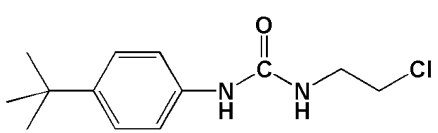
Finally, the natural product ottelione A (RPR112378) *(46)* inhibits tubulin polymerization and reacts with a single tubulin cysteine residue, which has thus far not been identified. Ottelione A also inhibits colchicine binding to tubulin and is a structural analog



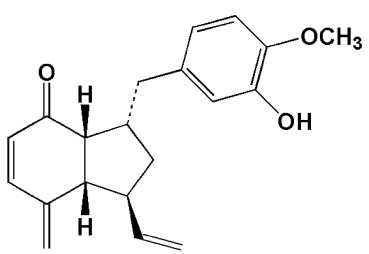
2-4-dichlorobenzyl thiocyanate



2-fluoro-1-methoxy-4-pentafluorophenylsulfonamidobenzene



4-*tert*-butyl-[3-(2-chloroethyl)ureido]benzene



Ottelione A

Fig. 3. Structures of antimitotic compounds that alkylate tubulin sulfhydryl group(s).

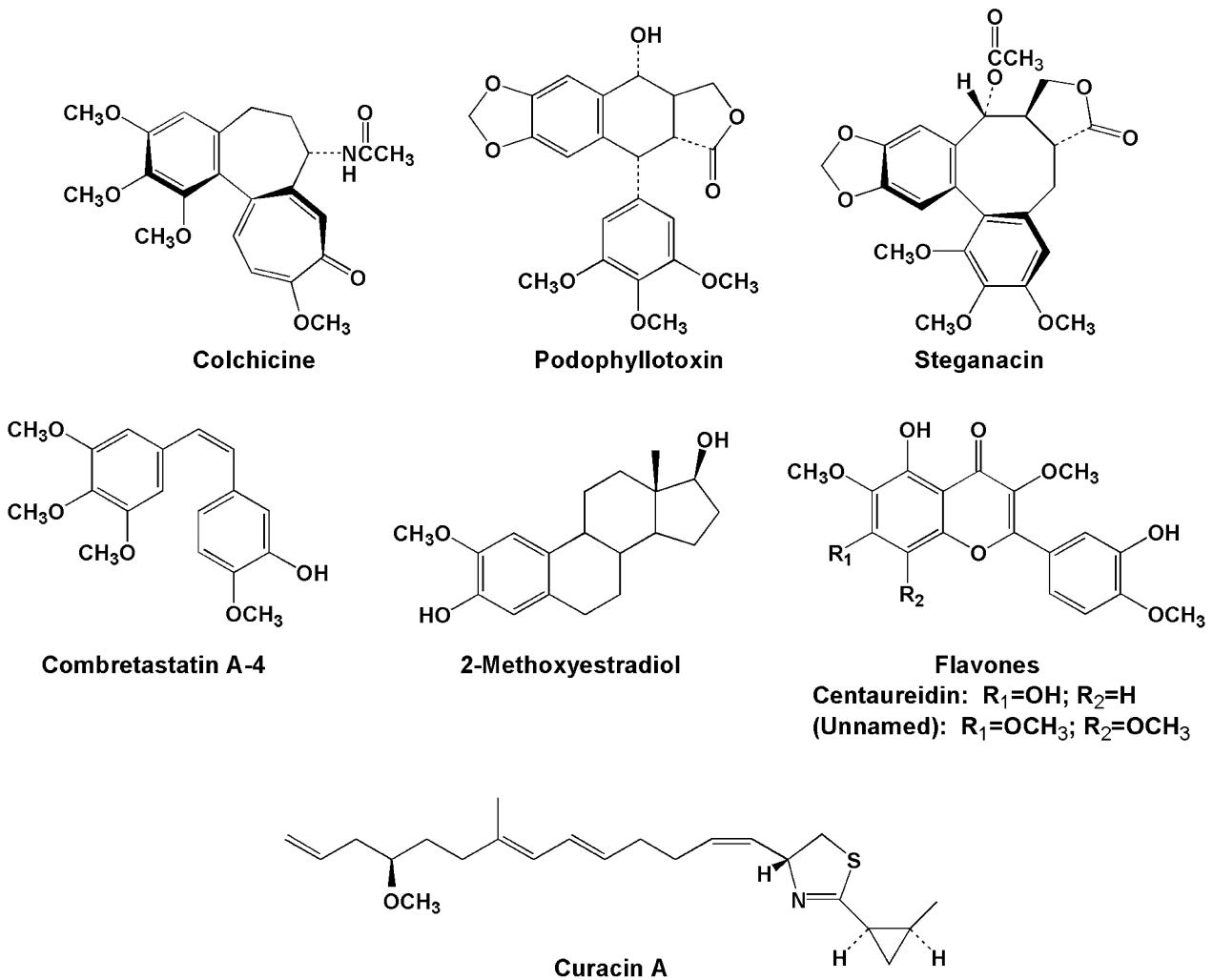


Fig. 4. Structures of natural products that bind in the colchicine site. The diagram of colchicine shows the (*aS*, *7S*) configuration proposed by Brossi and his collaborators *(105,106)*.

of colchicine site drugs. It is the most cytotoxic of the sulfhydryl reactive drugs, with an IC_{50} in human epithelial (KB) cells of 20 p*M*.

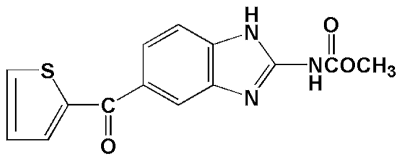
Unlike T138067 and ottelione A, most antimitotic drugs that inhibit colchicine binding to tubulin do not alkylate the protein. They seem to bind at a common site on β-tubulin close to the interface between the α- and β-subunits *(47)*, and colchicine analogs with chloroacetyl groups attached to the A ring alkylate both Cys-239 and Cys-354 of β-tubulin *(48,49)*. These compounds tend to be relatively simple structurally, compared with those inducing assembly or binding in the vinca domain, but are nevertheless structurally diverse. They include both natural products such as podophyllotoxin *(50)*, steganacin *(51)*, the combretastatins *(52)*, 2-methoxyestradiol *(53)*, flavones *(54–56)*, and the curacins *(57)* (examples are shown in Fig. 4) and a wide variety of synthetic compounds, such as carbamates *(58–60)*, heterocyclic ketones *(61)*, and benzoylphenylureas *(62)*. The examples of synthetic colchicine site compounds shown in Fig. 5 are only a small sample of the number of agents that have been reported. As inhibitors of cell growth, colchicine site drugs generally yield IC_{50}s in the low nanomolar to midmicromolar range.

Besides binding to unassembled αβ-tubulin dimer, colchicine, and assumedly other drugs that bind in the colchicine site, has a limited ability to enter microtubules. Small amounts of the tubulin–colchicine complex will copolymerize with tubulin free of drug, and the colchicine remains bound to the microtubules *(63,64)*. This would indicate that the colchicine site on tubulin is not entirely masked or obliterated in the polymer.

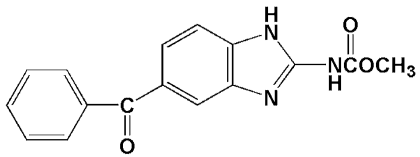
The only colchicine site ligand readily available in a radiolabeled form is colchicine itself. The binding reaction of colchicine to tubulin has a number of unusual properties *(65)* that most other drugs that bind at the colchicine site do not share. The binding interaction does not readily occur on ice, and becomes progressively more rapid at temperatures in the 20–40°C range. Once bound, the dissociation of colchicine from tubulin is extremely slow, so that the binding is sometimes described, incorrectly, as irreversible. In contrast, most of the colchicine site drugs with which the author's laboratory has worked bind readily to tubulin in the cold and readily dissociate. Thus, the extent of inhibition observed can be highly variable, depending not only on reaction time and temperature, but more importantly on the relative binding and dissociation rates of colchicine and the potential inhibitor. In at least two cases *(66,67)*, the author's laboratory initially concluded that a new class of synthetic molecules bound at a "new" site on tubulin, only to find that modification of reaction conditions *(68)* or synthesis of stronger analogs (unpublished data) revealed that the agents bound weakly to the colchicine site.

The last group of drugs with a relatively well-defined mechanism for interacting with tubulin bind in a region described as the "vinca domain." Almost all of these compounds inhibit the binding of vinblastine and vincristine to tubulin, but different inhibitory patterns are obtained when data are evaluated by the "classic" methods used in enzyme kinetics (Lineweaver-Burk and Hanes analyses). Both competitive and noncompetitive patterns occur. Thus, the author proposed that the vinca domain contains both the vinca site, where the competitive inhibitors bind, and nearby sites, where noncompetitive inhibitors bind. The strong inhibition observed with many noncompetitive inhibitors was postulated to derive from their interfering sterically with the vinca-binding site as a result of the close proximity of the different binding sites *(69)*.

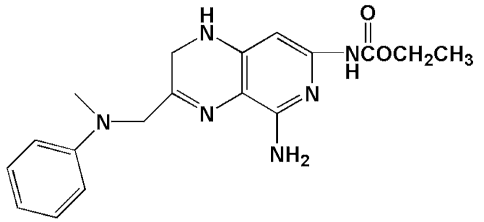
Drugs that bind in the vinca site itself are shown in Fig. 6. These include (1) the vinca alkaloids themselves, exemplified by vinblastine and vincristine, which competitively



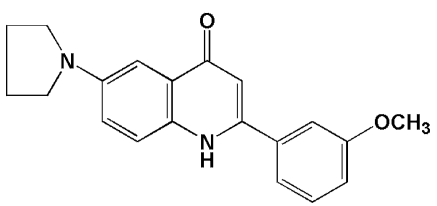
Nocodazole



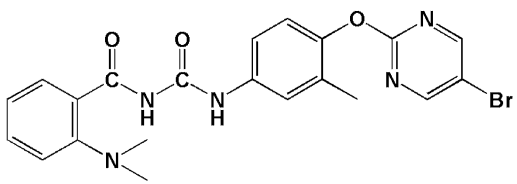
Mebendazole



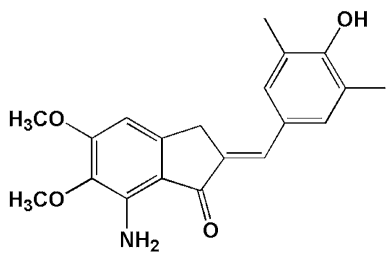
NSC 181928



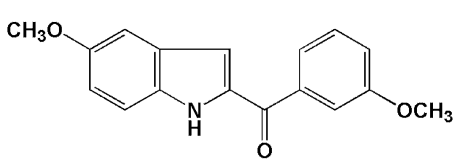
NSC 664171



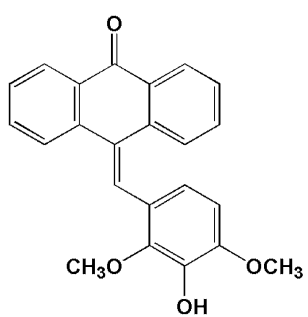
NSC 639829, a benzoylphenylurea



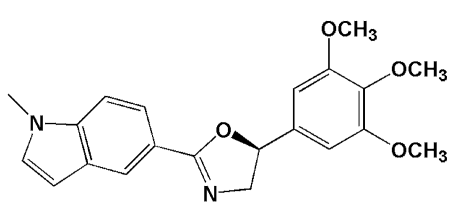
Indanocine



A 2-aroylindole

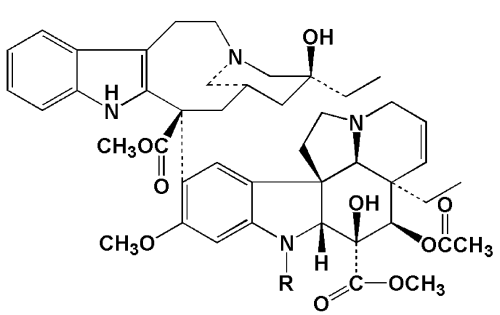


A benzylidene-9(10*H*)-anthracenone

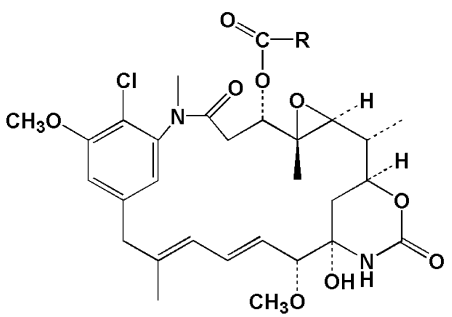


An indolyloxazoline

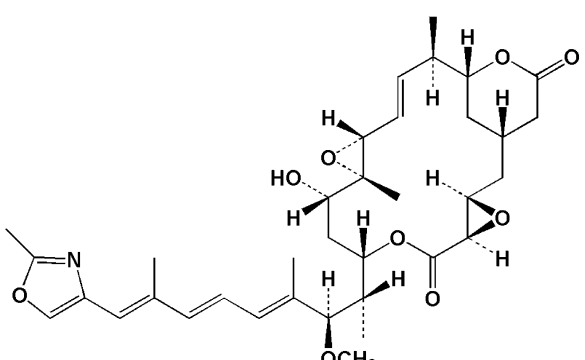
Fig. 5. Structures of synthetic compounds that inhibit the binding of colchicine to tubulin and thus probably bind in the colchicine site. Examples include the carbamates nocodazole, mebendezole, and NSC 181928 (first row); a potently cytotoxic heterocyclic ketone NSC 664171 *(61)*, a "second generation" benzoylphenylurea NSC 639829 in clinical trials, and indanocine *(107)* (middle row); a 2-aroylindole derivative *(108)*, a benzylidene-9(10*H*)-anthracenone derivative *(109)*, and a indolyloxazoline derivative *(110)* (bottom row).



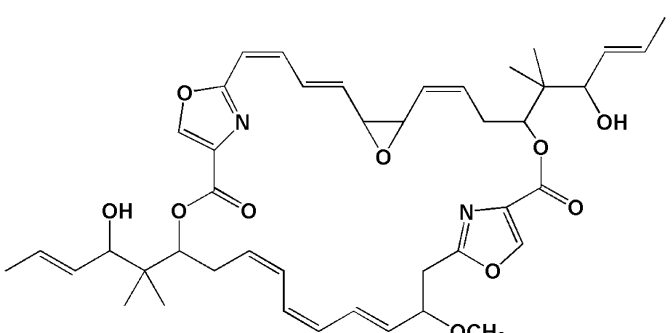
Vinblastine: R=CH_3
Vincristine: R=CHO



Maytansine: R=$CH(CH_3)N(CH_3)COCH_3$
Ansamitocin P-3: R=$CH(CH_3)_2$
Ansamitocin P-4: R=$CH_2CH(CH_3)_2$



Rhizoxin



Disorazol A_1

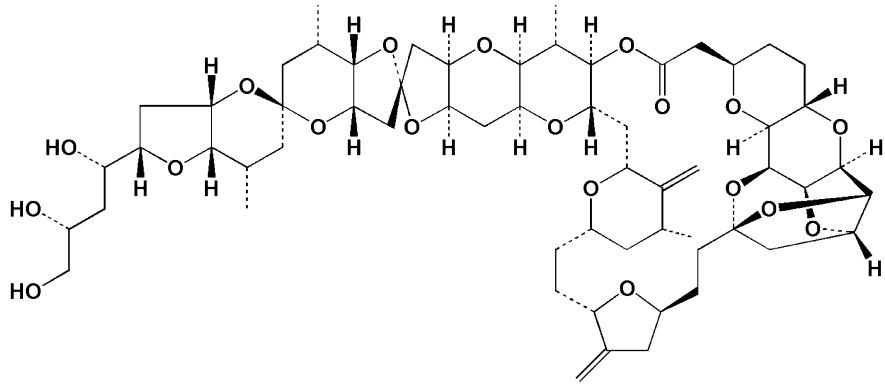
Fig. 6. Structures of natural products described as competitive inhibitors of the binding of vinblastine and/or vincristine to tubulin.

inhibit each other's binding to tubulin *(69,70)*; (2) the maytansinoids, exemplified by the plant product maytansine *(69,71,72)* and the fermentation products ansamitocins P-3 and P-4 *(73)*; (3) the fungal macrolide rhizoxin *(69)*; and (4) the myxobacterial macrocycle disorazol A_1 *(74–76)*. It should be noted that, in contrast to the competitive inhibition the author has observed with maytansine and rhizoxin vs vincristine or vinblastine *(69,72)*, Takahashi et al. *(77)* interpreted their binding data as indicating maytansinoids and rhizoxin bound at a common site on tubulin distinct from the vinca alkaloid-binding site. IC_{50}s of these compounds against a variety of cell lines range from 0.5 to 1 n*M* for maytansine to 20–40 n*M* for vinblastine. There is evidence that the vinca site, like the colchicine site, persists to a limited extent in microtubules, both at ends of microtubules and along their length. Binding of vinblastine to microtubules has been demonstrated to occur at a restricted number of αβ-tubulin dimers in the polymer *(78,79)*. The vinblastine site has recently been shown to be formed not by a single αβ-tubulin heterodimer but by the α-subunit of one heterodimer and the β-subunit of a second dimer *(80)*, consistent with the isodesmic tubulin assembly reaction induced by the drug *(81)* and with an early photoaffinity labeling study *(82)*.

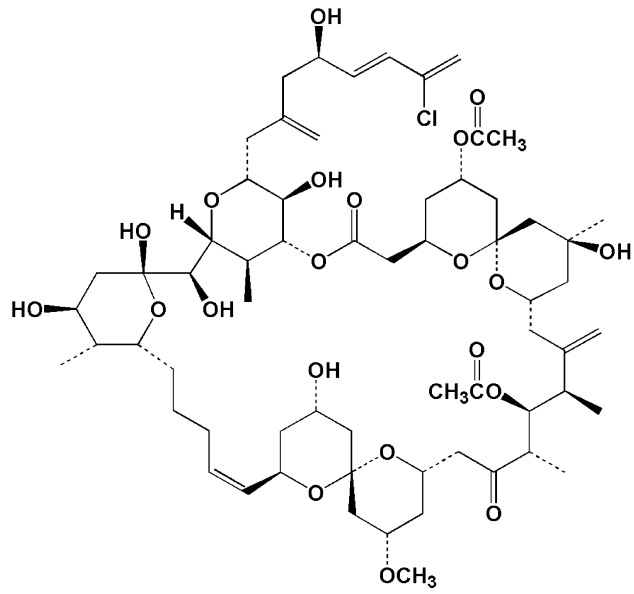
Noncompetitive inhibitors of vinca alkaloid binding to tubulin can be viewed as falling into two structural classes, macrocyclic polyethers and peptides/depsiptides. The former group is the smaller and consists of two families of complex molecules, the halichondrins *(72,83)* and the spongistatins *(70,84)*, the latter also known as the altohytrins *(85)*. The most thoroughly studied member of each of these families in terms of interactions with tubulin are halichondrin B *(72)* and spongistatin 1 *(70,84)*. These compounds are shown in Fig. 7, together with a simpler analog of halichondrin B, NSC 707389 *(86)*, which is presently in clinical trials as an anticancer agent. Both halichondrin B and spongistatin 1 inhibit the binding of radiolabeled vinblastine to tubulin in a noncompetitive manner *(70,72)*. In addition, spongistatin 1 is a noncompetitive inhibitor of the binding of one of the peptide antimitotics, dolastatin 10, to tubulin *(70)*. As dolastatin 10 is itself a noncompetitive inhibitor of vinca alkaloid binding to tubulin *(69)*, this suggests that the vinca domain may consist of at least three distinct drug-binding regions. The macrocyclic polyethers are highly cytotoxic, with low picomolar IC_{50}s obtained with the most potent spongistatins *(70)*.

The larger structural class of compounds that probably bind in the vinca domain is a structurally varied group of antimitotic peptides and depsipeptides. These molecules are all natural products, and their common feature is that they all contain highly modified amino acid residues. They are derived from a wide variety of organisms, and they display a wide range of cytotoxic activity (low micromolar IC_{50}s obtained with the phomopsins and ustiloxins; low picomolar IC_{50}s obtained with the most active cryptophycins and tubulysins—*see* ref. *87* for additional details). The peptides and depsipeptides will be divided here into two groups, those that have been shown to inhibit vinca alkaloid binding (Fig. 8) and those that have not (Fig. 9).

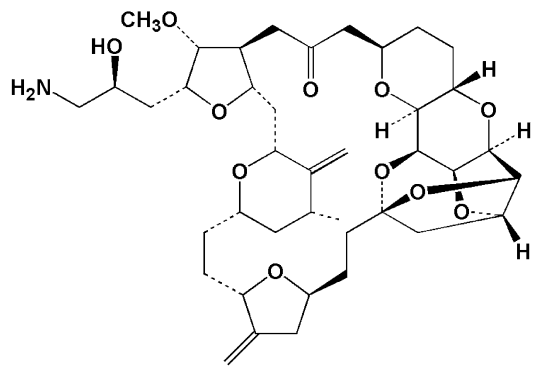
Strong inhibition of vinblastine, vincristine, and/or rhizoxin binding to tubulin has been observed with the fungus-derived agents phomopsin A *(88)* and ustiloxin A *(89)*; with the marine agents dolastatin 10 *(69)*, hemiasterlin *(90)*, and vitilevuamide *(91)*; with the cyanobacterial agent cryptophycin 1 *(92–94)*; and with the myxobacterial agent tubulysin A *(95)*. The related compound, tubulysin D, is more cytotoxic than tubulysin A *(96)*, and the absolute configuration of the former compound was recently established *(97)*.



Halichondrin B



Spongistatin 1



NSC 707389

Fig. 7. Structures of macrocyclic polyethers that inhibit the binding of vinblastine to tubulin.

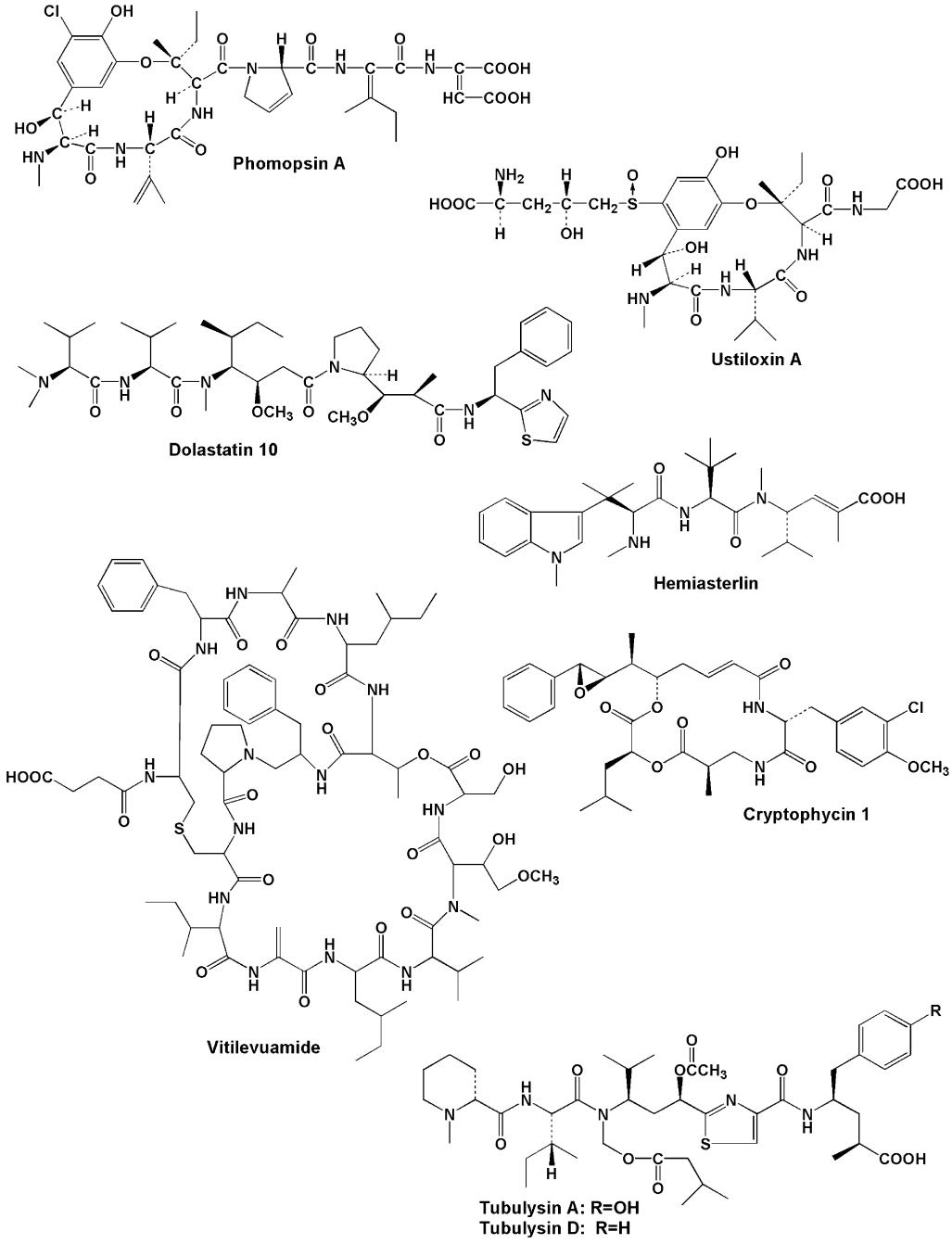
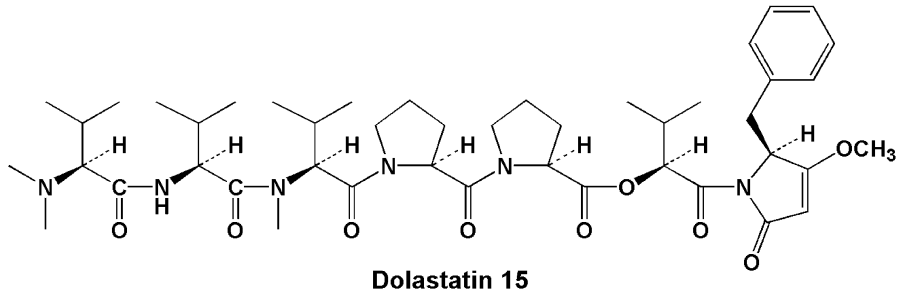
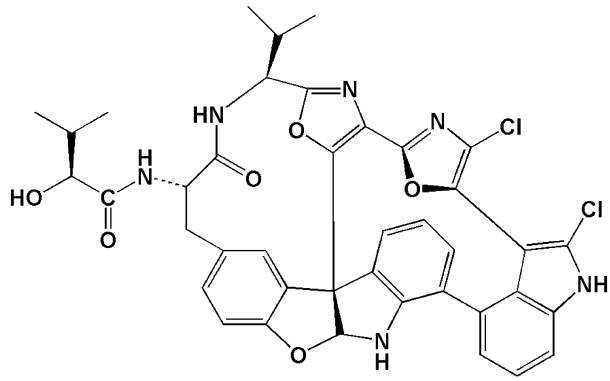


Fig. 8. Structures of peptides and depsipeptides that inhibit the binding of vinblastine and/or vincristine to tubulin. The configurations of the chiral centers for tubulysins A and D are as proposed by Höfle et al. *(97)* for tubulysin D.

For the structures shown in Fig. 8, the author has assumed that the chiral centers are identical in tubulysins A and D. Noncompetitive inhibition of vinblastine or vincristine binding by Lineweaver-Burk analysis or by the related Hanes analysis has been shown for phomopsin A *(69)*, dolastatin 10 *(69)* and a synthetic analog *(98)*, hemiasterlin *(90)*, vitilevuamide *(91)*, and cryptophycin 1 *(93)*.





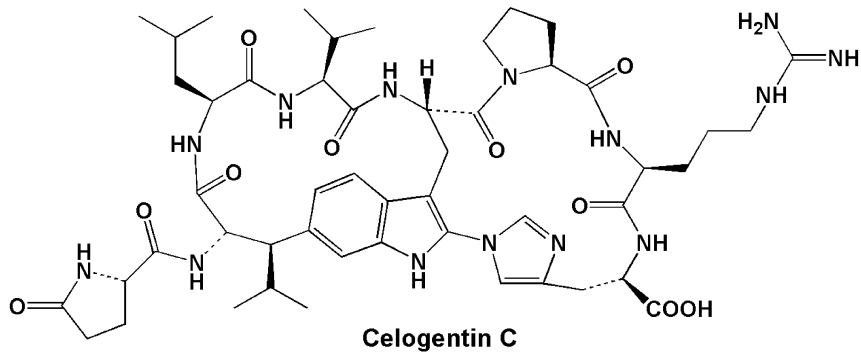


Fig. 9. Structures of a depsipeptide and two peptides that have not been reported to inhibit vinca alkaloid binding to tubulin or have been shown not to affect vinca alkaloid binding (*see* text).

The synthesis of radiolabeled dolastatin 10 *(99)* permitted ready evaluation of the interaction of an additional antimicrotubule drug with tubulin. Kinetic analysis of inhibitory effects of a number of vinca domain drugs has indicated that spongistatin 1 is a noncompetitive inhibitor of dolastatin 10 binding to tubulin *(70)*, whereas an analog of dolastatin 10 *(70)*, cryptophycin 1 *(93)*, hemiasterlin *(90)*, and phomopsin A (unpublished data) are competitive inhibitors.

Figure 9 shows the structures of three additional peptides and depsipeptides. Dolastatin 15 *(100)* and diazonamide A *(101)*, both marine products, have no apparent inhibitory effect on the binding of vinblastine to tubulin. Recent experiments with

radiolabeled dolastatin 15 demonstrated that the compound bound weakly to tubulin, with an apparent dissociation equilibrium constant of about 30 μ*M*. The binding of dolastatin 15 to tubulin was inhibited strongly by dolastatin 10, phomopsin A, halichondrin B, and maytansine; moderately by vinblastine and vincristine; weakly by cryptophycin 1; and not at all by a potent analog of dizaonamide A. These results suggest dolastatin 15 does bind weakly in the vinca domain but leave the binding site of diazonamide A still unknown *(102)*.

The last peptide shown in Fig. 9 is the plant product celogentin C *(103)*. It inhibits microtubule assembly and is the most potent inhibitor among a growing number of structurally related peptides. Thus far, no data have been published describing the effects of any of this group of peptides either on vinca alkaloid binding to tubulin or on cell growth.

4. SUMMARY

In summary, tubulin is the primary target of a large and ever-growing number of small molecules. Their mechanisms of interaction with the protein are varied. As new agents are discovered, the complexity of interactions of drugs with tubulin only seems to increase. In particular, the simultaneous binding of laulimalide and paclitaxel to polymer revealed an unexpected new dimension to drugs that induce tubulin assembly; and it is becoming increasingly more difficult to rationalize the binding of the structurally diverse antimitotic peptides and depsipeptides to a single, well-defined site on the tubulin molecule. Finally, cyclostreptin has been shown very recently to react covalently with two amino acid residues of β-tubulin (III), Thr–218 and Asn–226, providing new insights into the mechanism of drug binding to the taxoid site.

REFERENCES

1. Rowinsky EK, Donehower RC. Paclitaxel (Taxol). New Engl J Med 1995;332:1004–1014.
2. Cortes JE, Pazdur R. Docetaxel. J Clin Oncol 1995;13:2643–2655.
3. Aoe K, Kiura K, Ueoka H, et al. Effect of docetaxel with cisplatin or vinorelbine on lung cancer cells. Anticancer Res 1999;19:291–299.
4. Batra S, Karlsson R, Witt L. Potentiation by estramustine of the cytotoxic effect of vinblastine and doxorubicin in prostatic tumor cells. Int J Cancer 1996;68:644–649.
5. Budman DR, Calabro A. In vitro search for synergy and antagonism: evaluation of docetaxel combinations in breast cancer cell lines. Breast Cancer Res Treat 2002;74:41–46.
6. Budman DR, Calabro A, Kreis W. Synergistic and antagonistic combinations of drugs in human prostate cancer cell lines in vitro. Anti-cancer Drugs 2002;13:1011–1016.
7. Budman DR, Calabro A, Wang LG, et al. Synergism of cytotoxic effects of vinorelbine and paclitaxel in vitro. Cancer Invest 2000;18:695–701.
8. Carles G, Braguer D, Sabeur G, Briand C. The effect of combining antitubulin agents on differentiated and undifferentiated human colon cancer cells. Anti-cancer Drugs 1998;9:209–221.
9. Culine S, Roch I, Pinguet F, Romieu G, Bressolle F. Combination paclitaxel and vinorelbine therapy: in vitro cytotoxic interactions and dose-escalation study in breast cancer patients previously exposed to anthracyclines. Int J Oncol 1999;14:999–1006.
10. Giannakakou P, Villalba L, Li H, Poruchynsky M, Fojo T. Combinations of paclitaxel and vinblastine and their effects on tubulin polymerization and cellular cytotoxicity: characterization of a synergistic schedule. Int J Cancer 1998;75:57–63.
11. Knick V, Eberwein D, Miller CG. Vinorelbine tartrate and paclitaxel combinations: enhanced activity against in vivo P388 murine leukemia cells. J Natl Cancer Inst 1995;87:1072–1077.