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NATURAL PRODUCTS WHICH INTERACT WITH TUBULIN IN THE VINCA DOMAIN: MAYTANSINE, RHIZOXIN, PHOMOPSIN A, DOLASTATINS 10 AND 15 AND HALICHONDRIN B

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Abstract—This paper summarizes published data on the interactions of tubulin with antimitotic compounds that inhibit the binding of vinca alkaloids to the protein. These are all relatively complex natural products isolated from higher plants, fungi and marine invertebrate animals. These agents are maytansine, rhizoxin, phomopsin A, dolastatins 10 and 15 and halichondrin B and their congeners. Effects on tubulin polymerization, ligand binding interactions and structure–activity relationships are emphasized.

CONTENTS

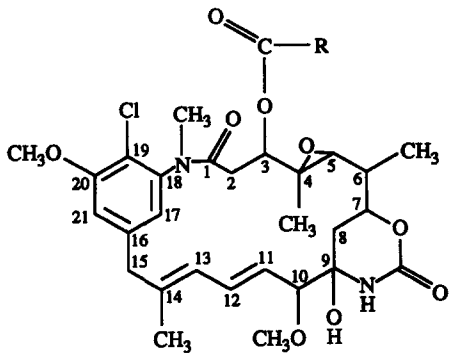
1. INTRODUCTION

Antimitotic agents almost universally alter microtubule assembly reactions. With the exception of taxol and its congeners (Schiff *et al.*, 1979), the primary effect of these compounds, the one observed at lower drug concentrations, is inhibition of the reaction. With the exception of estramustine and its phosphate derivative (Stearns and Tew, 1985; Wallin *et al.*, 1985), these inhibitors all interact with tubulin, the predominant protein component of microtubules. Although tubulin molecules are not uniform, since there is minor primary structure variability (Krauhs *et al.*, 1981; Ponstingl *et al.*, 1981) and substantial variability resulting from post-translational modification (Eipper, 1972; Arce *et al.*, 1975; Maruta *et al.*, 1986; Eddé *et al.*, 1990; Alexander *et al.*, 1991), each tubulin unit in a microtubule consists of two highly similar polypeptide chains (α- and β-tubulin) and two guanosine nucleotides. The two polypeptide chains are nearly identical in molecular weight (about 50 kDa) and have approximately 40% absolute sequence homology and about 70% homology if conservative amino acid substitutions are considered (Krauhs *et al.*, 1981; Ponstingl *et al.*, 1981). One guanosine nucleotide is in the form of GDP in the microtubule while in unpolymerized tubulin it can be in the form of either GDP or GTP. This nucleotide is

exchangeable with GDP or GTP in solution in unpolymerized tubulin (Weisenberg *et al.*, 1968; Kobayashi, 1974; Levi *et al.*, 1974; Caplow and Zeeberg, 1980; Lin and Hamel, 1987), but it becomes inaccessible in polymerized tubulin (Weisenberg *et al.*, 1976; Arai and Kaziro, 1977; David-Pfeuty *et al.*, 1977). The exchangeable nucleotide has been localized to the β-subunit of tubulin (Geahlen and Haley, 1977; Nath *et al.*, 1985). The second guanosine nucleotide is always in the form of GTP and is always inaccessible to solution nucleotides. Even in cells its half-life appears to be equal to that of tubulin itself (Spiegelman *et al.*, 1977). The location of the nonexchangeable nucleotide has not been determined, but the sequence homology of the tubulin subunits suggests it may be on α-tubulin.

Inhibitors of microtubule assembly fall into two broad classes, which have been essentially defined by their effects on the binding to tubulin of two commercially available radiolabeled drugs. Most inhibitors of polymerization, including virtually all synthetic compounds, inhibit the binding of radiolabeled colchicine to tubulin and do not affect the binding of radiolabeled vinblastine to tubulin. Generally, despite rather diverse but relatively uncomplicated molecular structures, when carefully examined such compounds show a competitive pattern of inhibition against colchicine.

Inhibitors of radiolabeled vinblastine binding are much less common. Thus far, all such inhibitors are natural products (or closely related synthetic analogs) and their structures are complex. These agents are the subject of this paper. None of them inhibit the binding of radiolabeled colchicine to tubulin. Their effects on vinblastine and vincristine binding fall into both competitive and noncompetitive patterns. In addition, all interfere with interactions of guanosine nucleotides at the exchangeable site. Finally, while no colchicine-site drug has a role in the treatment of neoplastic diseases, the efficacy of the vinca alkaloids in cancer chemotherapy (see Rowinsky and Donehower, 1992) lends particular interest to the diverse group of natural products that interfere with the tubulin–vinblastine interaction.



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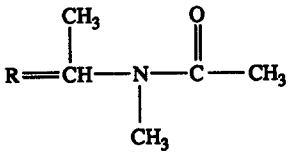

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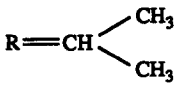

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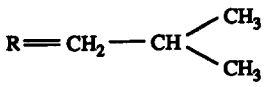

FIG. 1. Structural formulae of maytansine, ansamitocin P-3 and ansamitocin P-4.

2. THE MAYTANSINOIDS

Maytansine (NSC 153858; structure presented in Fig. 1) is the most thoroughly studied member of a series of ansa macrolide compounds isolated and characterized by Kupchan and his collaborators (Kupchan *et al.*, 1972, 1974, 1975, 1977, 1978). These agents were obtained from the plants *Maytenus ovatus*, *M. buchananii*, *M. serrata* and the related *Putterlickia verrucosa*. Chemically related compounds have also been isolated from *Colubrina texensis*, a member of a different plant family (Wani *et al.*, 1973) and from a *Nocardia* species (Higashide *et al.*, 1977; Asai *et al.*, 1979). The best studied compounds from the microorganism are ansamitocin P-3 and ansamitocin P-4 (structures in Fig. 1). Maytansine (Remillard *et al.*, 1975; Wolpert-DeFilippes *et al.*, 1975a,b; Kupchan *et al.*, 1978; Bai *et al.*, 1990b) and the ansamitocins (Higashide *et al.*, 1977; Ootsu *et al.*, 1980) are highly cytotoxic compounds (see below) which inhibit mitosis, with cells accumulating in apparent metaphase arrest (condensed chromosomes; absent nuclear membrane; no spindle or poorly defined spindle with few if any microtubules). Except for taxol (Rowinsky and Donehower, 1992), maytansine is the only antimitotic agent to have gone through extensive clinical trials for the treatment of neoplastic diseases (e.g. Cabanillas *et al.*, 1979; Rosenthal *et al.*, 1980), but thus far no useful role for the drug has been established in clinical practice. Recently maytansine derivatives have been coupled to tumor-specific antibodies in a new approach to clinical use of this agent (Chari *et al.*, 1992).

Radiolabeled maytansine binds to tubulin in a reversible reaction which is relatively fast, occurs at 0°C and is inhibited by vincristine (Mandelbaum-Shavit *et al.*, 1976) and vinblastine (Batra *et al.*, 1986). The apparent K_d value for the reaction at 37°C is 0.7 μM (Mandelbaum-Shavit *et al.*, 1976). Vincristine was found to act as a competitive inhibitor of maytansine with an apparent K_i value of 10 μM (Mandelbaum-Shavit *et al.*, 1976). Neither vincristine (Mandelbaum-Shavit *et al.*, 1976) nor vinblastine (Batra *et al.*, 1986) displaces all radiolabeled maytansine bound to tubulin. This phenomenon has not been studied in detail, but may be related to observations of Takahashi *et al.* (1987b) on apparently contradictory effects of vinblastine on the binding of radiolabeled rhizoxin to tubulin (competitive) as compared with the effects of rhizoxin on the binding of radiolabeled vinblastine (not purely competitive) (see below).

In contrast, nonradiolabeled maytansine almost totally displaces radiolabeled vinblastine or vincristine from tubulin (Mandelbaum-Shavit *et al.*, 1976; Batra *et al.*, 1986; Safa *et al.*, 1987). Maytansine has been found to competitively inhibit the binding of both vinca agents to tubulin. Apparent K_i values vs vincristine have been reported as 0.4 μM (Mandelbaum-Shavit *et al.*, 1976; York *et al.*, 1981) and 3.1 μM (Bai *et al.*, 1990a) and versus vinblastine as 0.5 μM (Bhattacharyya and Wolff, 1977) and 0.9 μM (Bai *et al.*, 1991). Lacey *et al.* (1987) found that the IC_{50} value of maytansine versus radiolabeled vinblastine was 1.6 μM. All workers have concluded that maytansine binds to tubulin with greater affinity than these two vinca agents. In addition, ansamitocin P-3 inhibits the binding of radiolabeled vinblastine to tubulin, but the data indicated that the inhibition was not competitive (Takahashi *et al.*, 1987b).

Maytansine, like vinblastine, has only minor effects on the binding of radiolabeled colchicine to tubulin (Mandelbaum-Shavit *et al.*, 1976; Batra *et al.*, 1986; Lacey *et al.*, 1987). Unlike vinblastine, however, maytansine does not stabilize the colchicine binding activity of tubulin (Bhattacharyya and Wolff, 1977; Bai *et al.*, 1990a). Tubulin stabilization by vinblastine, but not maytansine, has also been demonstrated by the ability of the former, but not the latter, to reduce the rate at which bis(8-anilinonaphthalene-1-sulfonate) interacts nonspecifically with denatured hydrophobic regions of tubulin (Prasad *et al.*, 1986).

Maytansine has specific effects on the alkylation of tubulin sulfhydryl groups, which have been studied by Ludueña and his colleagues (Ludueña and Roach, 1981a,b; Ludueña *et al.*, 1982; Roach and Ludueña, 1984). This work has been reviewed in detail in this series (Ludueña and Roach, 1991). In brief, these workers have evaluated the effects of a large variety of tubulin ligands, including many antimitotic agents, on the pattern of alkylation observed with iodoacetamide and on the formation of intrapolypeptide chain crosslinks in β-tubulin following reaction with the bifunctional agent *N,N'*-ethylenebis(iodoacetamide). There are two potential major crosslinks. The first, between cys 239 and cys 354 (Little and Ludueña, 1985) (referred to throughout this paper as the 'first crosslink'), occurs in all tubulin preparations. The second,

between cys 12 and either cys 201 or cys 211 (Little and Ludueña, 1987) (referred to as the 'second crosslink'), only occurs in nucleotide-depleted tubulin with its formation strongly inhibited by GTP. Maytansine had minimal effects on alkylation by iodoacetamide, in contrast to strong inhibition by vinblastine. Formation of the first crosslink was enhanced in the presence of both maytansine and vinblastine, while formation of the second crosslink was almost completely inhibited by maytansine and weakly inhibited by vinblastine. Since the exchangeable nucleotide site has been localized to the β-subunit, this finding suggests that the maytansine binding site may also be on β-tubulin.

Our own studies also led to this conclusion. Maytansine potently inhibits tubulin-dependent GTP hydrolysis (Lin and Hamel, 1981; Bai *et al.*, 1990b) and nucleotide binding to tubulin, particularly at lower reaction temperatures (Huang *et al.*, 1985; Bai *et al.*, 1990a, 1991). Maytansine does not displace nucleotide bound in the exchangeable site (Huang *et al.*, 1985; Bai *et al.*, 1990a), and, moreover, the drug prevents nucleotide loss from tubulin during gel filtration chromatography (Lin and Hamel, 1987). The most reasonable explanation for these observations is that maytansine binds to β-tubulin in a manner that prevents entry and exit of guanosine nucleotides at the exchangeable site. We also have evaluated vinblastine as an inhibitor of nucleotide exchange on tubulin (Huang *et al.*, 1985; Bai *et al.*, 1990a, 1991). Although the vinca alkaloid has some effect on the reaction, significant inhibition is only observed at high concentrations (at least 100 μM).

In contrast to results from the alkylation and GTP exchange experiments with maytansine, however, are genetic results. The only maytansine-resistant mutant reported thus far has been a Chinese hamster ovary cell line with an altered α-tubulin (Schibler and Cabral, 1985). Takahashi *et al.* (1990) have, however, described an interesting manipulation by site-directed mutagenesis in a β-tubulin gene of *Schizosaccharomyces pombe*. Ileu 100 was converted to asn 100 (analogous to the situation in mammalian β-tubulin) and an organism with increased sensitivity to ansamitocin P-3, as well as rhizoxin (see below), was produced.

Maytansine and the ansamitocins inhibit microtubule assembly requiring microtubule-associated proteins (Remillard *et al.*, 1975; Bhattacharyya and Wolff, 1977; Kupchan *et al.*, 1978; Ootsu *et al.*, 1980; York *et al.*, 1981; Fellous *et al.*, 1985; Huang *et al.*, 1985; Lacey *et al.*, 1987; Takahashi *et al.*, 1987a) and the glutamate-induced polymerization of purified tubulin (Bai *et al.*, 1990b). With microtubule-associated proteins, a higher concentration of maytansine is required with microtubule-associated protein 2 as compared with tau factor to obtain equivalent inhibition (Fellous *et al.*, 1985). With the exception of one report where an IC_{50} value for polymerization of 0.4 μM was obtained (Bhattacharyya and Wolff, 1977), most workers have obtained IC_{50} values for maytansine and the ansamitocins in the 1–5 μM range (Remillard *et al.*, 1975; Ootsu *et al.*, 1980; Huang *et al.*, 1985; Lacey *et al.*, 1987; Takahashi *et al.*, 1987a; Bai *et al.*, 1990b). Despite binding more avidly to tubulin than vinblastine (see above), in direct comparisons maytansine (and the ansamitocins) has always been found to be less effective than vinblastine as an inhibitor of polymerization (Bhattacharyya and Wolff, 1977; Ootsu *et al.*, 1980; Huang *et al.*, 1985; Lacey *et al.*, 1987; Takahashi *et al.*, 1987a; Bai *et al.*, 1990b).

Concentrations of both maytansine and the ansamitocins below 20 μM cause extensive disassembly of preformed microtubules (Remillard *et al.*, 1975; Ootsu *et al.*, 1980) and the ansamitocins cause intracellular microtubules to disappear (Ootsu *et al.*, 1980).

Unlike the vinca alkaloids (reviewed by Himes, 1991), neither maytansine nor the ansamitocins induce formation of spiral aggregates of tubulin (Bhattacharyya and Wolff, 1977; Ootsu *et al.*, 1980; Fellous *et al.*, 1985; Takahashi *et al.*, 1987a). Instead maytansine potently inhibits this reaction, for maytansine at concentrations substoichiometric to those of both tubulin and vinblastine (e.g. 2 μM maytansine, 10 μM tubulin, 140 μM vinblastine) totally prevents tubulin spiralization (Fellous *et al.*, 1985). In addition, maytansine causes dissolution of preformed vinblastine-induced aggregates (Fellous *et al.*, 1985).

There is minimal information about tubulin interactions with any maytansinoids other than the ansamitocins, which differ solely in the ester substituent at position C(3) (see Fig. 1). York *et al.* (1981) examined four analogs as inhibitors of vincristine binding to tubulin. A compound with an altered ester substituent at position C(3) was essentially equivalent to maytansine, but three analogs with no substituent at this position and a 2–3 double bond were significantly less potent as inhibitors. One of these (which also lacked the *N*-methyl group and the 4–5 epoxide) had an

apparent noncompetitive pattern of inhibition, but its K_i value was 5 μM as opposed to the 0.4 μM value obtained for maytansine. Kupchan *et al.* (1978) obtained initial results with a large series of analogs. They compared inhibitory effects on microtubule assembly, mitosis of fertilized sea urchin eggs and growth of KB carcinoma cells in culture. There was little correlation between effects observed in the three assays and they only described compounds with significant inhibitory effects on the assembly reaction. The following modifications (see Fig. 1) did not seem to reduce maytansine's effectiveness as an inhibitor of assembly: altered ester at position C(3), hydroxyl group at position C(3), etherification of the hydroxyl at position C(9), thioether instead of hydroxy group at position C(9), no substituent at position C(3) together with introduction of a 2–3 double bond (in contrast to the finding of York *et al.*, 1981), no *N*-methyl group and acetyl group at position C(15).

The crystal structure of maytansine 3-bromopropyl ether has been summarized (Kupchan *et al.*, 1972, 1974; Bryan *et al.*, 1973). Substituents are oriented in a manner that minimizes intramolecular repulsions. The two longer sides of the 19-member ring are approximately parallel and about 5.4 Å apart. The ring appears to be open in its center. The face of the ring with the ester group is relatively hydrophilic, the opposite face hydrophobic.

3. RHIZOXIN

The fungus *Rhizopus chinensis* is the etiologic agent for a disease known as rice seedling blight in which there is an abnormal swelling of plant roots secondary to the failure of cell division (Ibaragi, 1973). A number of compounds which reproduced the disease process were isolated and characterized by Iwasaki and coworkers (Iwasaki *et al.*, 1984, 1986a,b; Kobayashi *et al.*, 1986). The most important of these agents was called rhizoxin (NSC 332598; structure in Fig. 2). The most prominent structural feature of rhizoxin is its 16-member macrolide ring. Besides its toxicity for plant tissues, rhizoxin also has antitumor (Tsuruo *et al.*, 1986) and antifungal (Iwasaki *et al.*, 1984) activity and it causes the accumulation of cells arrested in mitosis (Tsuruo *et al.*, 1986; Bai *et al.*, 1990b). Rhizoxin is in the early stages of clinical evaluation in human cancer patients (Bisset *et al.*, 1992).

Radiolabeled rhizoxin binds rapidly to tubulin at 37°C and the reaction is reversible. Scatchard analysis of binding data indicated one high affinity binding site with a K_d value of 0.2 μM (Takahashi *et al.*, 1987b). Binding of the drug to tubulin was inhibited by both vinblastine and ansamitocin P-3 (Takahashi *et al.*, 1987b). Lineweaver–Burk analysis indicated that both inhibitors were acting in a competitive manner, with apparent K_i values of 0.1 μM for ansamitocin P-3 and 3 μM for vinblastine (Takahashi *et al.*, 1987b). The binding of radiolabeled rhizoxin to tubulin is also strongly inhibited by phomopsin A (Li *et al.*, 1992). The IC_{50} value obtained with 3 μM rhizoxin was just over 0.1 μM for phomopsin A, but the type of inhibition (competitive or noncompetitive) was not defined in this study.

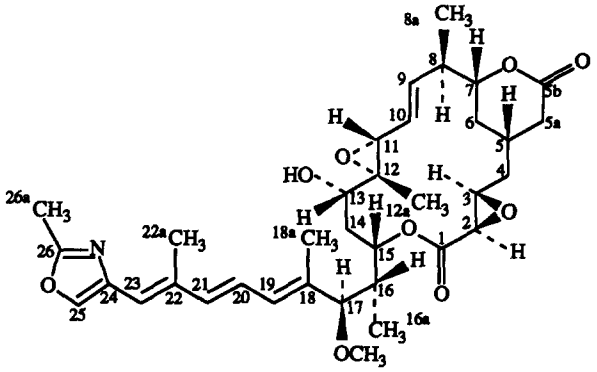

FIG. 2. Structural formula of rhizoxin.

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