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DOLASTATIN 15, A POTENT ANTIMITOTIC DEPSIPEPTIDE DERIVED FROM *DOLABELLA AURICULARIA*

INTERACTION WITH TUBULIN AND EFFECTS ON CELLULAR MICROTUBULES

RUOLI BAI,* SUSAN J. FRIEDMAN,† GEORGE R. PETTIT‡ and ERNEST HAMEL*§

*Laboratory of Molecular Pharmacology and †Laboratory of Biological Chemistry, Developmental Therapeutics Program, Division of Cancer Treatment, National Cancer Institute, National Institutes of Health, Bethesda, MD 20892; and ‡Cancer Research Institute and Department of Chemistry, Arizona State University, Tempe, AZ 85287, U.S.A.

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Abstract—Dolastatin 15, a seven-subunit depsipeptide derived from *Dolabella auricularia*, is a potent antimitotic agent structurally related to the antitubulin agent dolastatin 10, a five-subunit peptide obtained from the same organism. We have compared dolastatin 15 with dolastatin 10 for its effects on cells grown in culture and on biochemical properties of tubulin. The IC_{50} values for cell growth were obtained for dolastatin 15 with L1210 murine leukemia cells, human Burkitt lymphoma cells, and Chinese hamster ovary (CHO) cells (3, 3, and 5 nM with the three cell lines, respectively). For dolastatin 10, IC_{50} values of 0.4 and 0.5 nM were obtained with the L1210 and CHO cells, respectively. At toxic concentrations dolastatin 15 caused the leukemia and lymphoma cells to arrest in mitosis. In the CHO cells both dolastatin 15 and dolastatin 10 caused moderate loss of microtubules at the IC_{50} values and complete disappearance of microtubules at concentrations 10-fold higher. Despite its potency and the loss of microtubules in treated cells, the interaction of dolastatin 15 with tubulin *in vitro* was weak. Its IC_{50} value for inhibition of glutamate-induced polymerization of tubulin was 23 μM, as compared to values of 1.2 μM for dolastatin 10 and 1.5 μM for vinblastine. Dolastatin 10 noncompetitively inhibits the binding of vincristine to tubulin, inhibits nucleotide exchange, stabilizes the colchicine binding activity of tubulin, and inhibits tubulin-dependent GTP hydrolysis (Bai *et al.*, *Biochem Pharmacol* **39**: 1941–1949, 1990; Bai *et al. J Biol Chem* **265**: 17141–17149, 1990). Only the latter reaction was inhibited by dolastatin 15. Nevertheless, its structural similarity to dolastatin 10 indicates that dolastatin 15 may bind weakly in the "vinca domain" of tubulin (a region of the protein we postulate to be physically close to but not identical with the specific binding site of vinca alkaloids and maytansinoids), presumably in the same site as dolastatin 10 (the "peptide site").

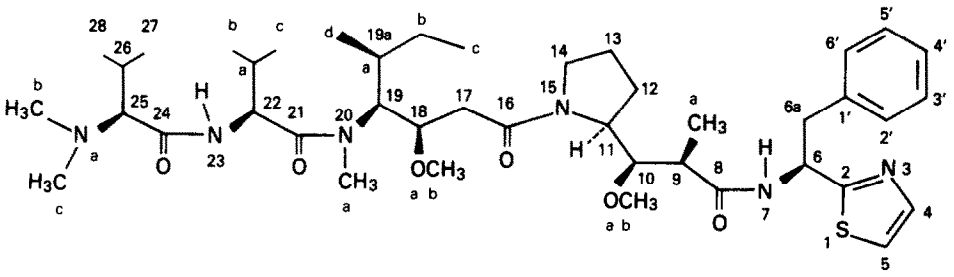
The shell-less mollusk *Dolabella auricularia* has yielded a number of peptides and depsipeptides composed largely of unusual amino and hydroxy acids and amino acid derivatives [1–6]. Most of these compounds have significant cytotoxic activity and potential as antineoplastic agents. Equally important, because they are oligomers of simpler subunits, practical syntheses of all these compounds are realistic goals.

Our initial work focused on dolastatin 10 (structure shown in Fig. 1), which was chosen both because of its relatively simple structure and its high toxicity for murine leukemia cells [7–9]. We found that natural dolastatin 10 is a potent inhibitor of tubulin polymerization and arrested leukemia cells in mitosis [7]. When synthetic dolastatin 10 became available [10], we examined its mechanism of action in some detail, including a structure–activity comparison with a number of synthetic isomers altered at one or more of its chiral centers [9]. We found that dolastatin 10 is a potent noncompetitive inhibitor of the binding of vinca alkaloids to tubulin, that it strongly interferes with nucleotide exchange at the exchangeable GTP site on β-tubulin, and that it effectively stabilizes the colchicine binding activity of tubulin [8].

The recent chemical synthesis of dolastatin 15 [11] (structure shown in Fig. 1) has now permitted us to undertake analogous studies with this compound. Structurally, dolastatin 15 is composed of seven subunits, five of which are amino acids. Two of these are modified valine residues, and the *N,N*-dimethylvaline at the amino terminus, called dolavaline, is unique to *D. auricularia*. The sixth and seventh subunits (2-hydroxyisovaleric acid and a complex amide, named dolapyrrolidone, probably derived from phenylalanine) are esterified to the amino terminal pentapeptide. Dolastatin 15 is similar to dolastatin 10 in the dolavaline–valine sequence at its amino terminus and in the aromatic character of its carboxyl terminus. Dolastatin 15, however, differs from dolastatin 10 in having an ester linkage in its unit bonding, and is thus a depsipeptide. In addition, dolastatin 15 lacks the dolaisoleuine residue which appears to be critical for the high activity of dolastatin 10 as an inhibitor of tubulin polymerization [9]. Nonetheless, since configuration at position 19a in

§ Corresponding author: Dr. E. Hamel, Building 37, Room 5C25, National Institutes of Health, Bethesda, MD 20892. Tel. (301) 496-4855; FAX (301) 496-5839.

DOLASTATIN 10 (NSC 376128)



DOLASTATIN 15 (NSC 617668)

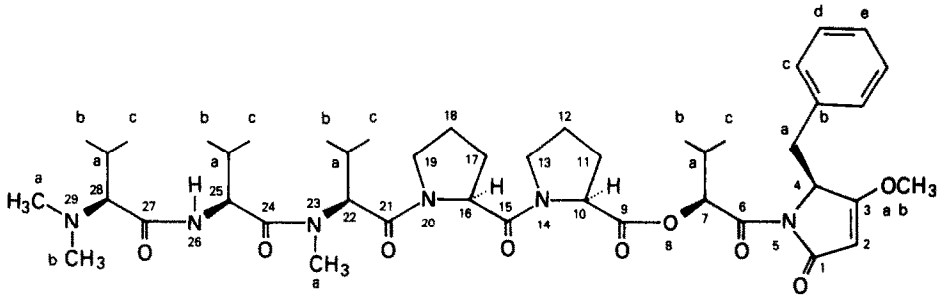


Fig. 1. Structural formulae of dolastatin 10 (NSC 376128) and dolastatin 15 (NSC 617668).

the dolaisoleuine side chain does not alter the potency of dolastatin 10 [9], the structural analogy, from the amino terminus, of dolastatin 15 to dolastatin 10 probably extends as far as the carbons bearing the side chain substituents [C(19) in dolastatin 10, C(22) in dolastatin 15].

As with dolastatin 10, preliminary work with the natural compound demonstrated that dolastatin 15 inhibited tubulin polymerization and caused cells to accumulate in mitosis. While not as potent as dolastatin 10, IC_{50} values for dolastatin 15 were reproducibly in the low nanomolar range. The compound thus merited careful evaluation, and the synthesis of adequate amounts of dolastatin 15 [11] made possible the studies presented here.

MATERIALS AND METHODS

Materials. Electrophoretically homogeneous bovine brain tubulin was prepared as described previously, including gel filtration chromatography of the tubulin to remove unbound nucleotide [12]. Nonradiolabeled GTP and [8-^{14}C]GTP were obtained from Sigma and Moravek Biochemicals, respectively, and repurified by triethylammonium bicarbonate gradient chromatography on DEAE Sephadex A-25. Monoclonal murine anti-β-tubulin antibody was from Sigma, and goat F(ab')$_2$-antimouse IgG conjugated with Texas red was from Tago. CHO* cells were obtained from the American Type Culture Collection. F-12 nutrient medium and fetal bovine serum were from Gibco. Nonradiolabeled vinblastine and colchicine were from Sigma and [^{3}H]vinblastine and [^{3}H]colchicine from Amersham. Rhizoxin was provided by the Drug Synthesis and Chemistry Branch, National Cancer Institute, and dolastatin 10 was synthesized as described previously [10]. Natural dolastatin 15 was obtained from *D. auricularia* [5], and synthetic dolastatin 15 was prepared as described elsewhere [11]. The synthetic depsipeptide was used in all studies presented here except as indicated. All drugs were dissolved in dimethyl sulfoxide, and control reaction mixtures contained concentrations of the solvent equivalent to those in the reaction mixtures containing drugs.

* Abbreviations: CHO, Chinese hamster ovary; PBS, phosphate-buffered saline (pH 7.2); Pipes, 1,4-piperazineethanesulfonate; EGTA, ethylene glycol bis(β-aminoethyl ether)-*N,N,N',N'*-tetraacetate; DAPI, 4,6-diamidino-2-phenylindole; and MAPs, microtubule-associated proteins.

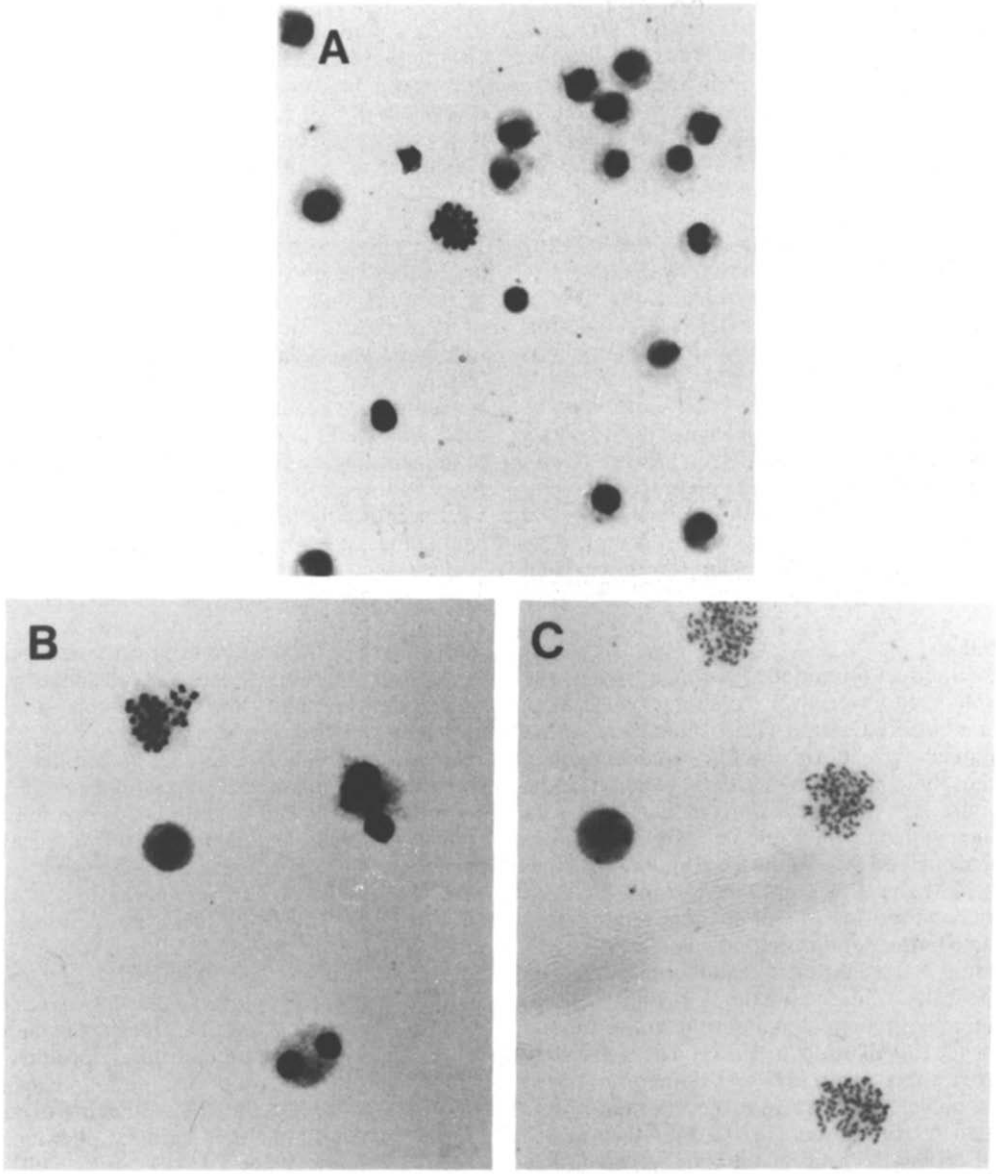


Fig. 2. Effect of dolastatin 15 on the morphology of L1210 murine leukemia cells. (A) Untreated cells. (B) Cells treated with 10 nM dolastatin 15. (C) Cells treated with 100 nM dolastatin 15. Magnification: ×300.

Methods. Tubulin polymerization was followed turbidimetrically in Gilford recording spectrophotometers equipped with electronic temperature controllers. Experimental detail and determination of IC_{50} values for drugs, based on inhibition of the extent of polymerization following a 15-min drug–tubulin preincubation in the absence of GTP and a 20-min incubation at 37° after GTP addition, have been described in detail [13]. Methodologies for measuring radiolabeled drug or GTP binding to tubulin by DEAE-cellulose filters or centrifugal gel filtration were described previously [14, 15], as was the quantitation of GTP hydrolysis by determining the amount of [8-^{14}C]GDP formed from [8-^{14}C]GTP by thin-layer chromatography on polyethyleneimine-cellulose and autoradiography [15].

Techniques for culturing L1210 murine leukemia cells and determining the mitotic index were described previously [16]. The suspension cultures contained 0.1% (v/v) dimethyl sulfoxide. Since cell number did not decrease over the incubation times used here, IC_{50} values are defined as the concentration of drug required to inhibit increase in cell number during incubation by 50% relative to the increase in the control cultures (i.e. the concentration to obtain $100 \times \{[\text{number of drug-treated cells} - \text{number of control cells at zero time}]/[\text{number of control cells} - \text{number of control cells at zero time}]\} = 50$). The

effects of dolastatin 10 and dolastatin 15 on cellular microtubules were examined in wild type CHO cells, with an initial evaluation of the effects of the two agents on the growth of this cell line. Cells were seeded at less than 20% confluence in Lab-Tek 8-chamber tissue culture glass slides (from Nunc) for immunofluorescence studies or in 96-well dishes for determination of IC_{50} values. The cells were grown in F-12 nutrient medium supplemented with 10% fetal bovine serum at 37° for 20 hr prior to drug addition. The cell plating density was chosen to ensure that cell growth was optimal at the time of drug addition. Medium was changed in all cultures at the time of drug addition, and incubation was continued for 16 hr at 37°.

Cell growth was measured by the sulforhodamine B protein assay described by Skehan *et al.* [17], with A_{564} values for the individual wells measured with a Dynatech model MR580 microplate reader. Over the time period examined, no net loss of protein occurred at the highest drug concentrations examined. The IC_{50} values were therefore determined by a formula analogous to that described above for determination of IC_{50} values for L1210 cell proliferation.

For the indirect immunofluorescence studies, the CHO cell monolayers were washed twice with phosphate-buffered saline (PBS) and fixed either with methanol at −20° or with 4% formaldehyde in 0.1 M phosphate buffer (pH 7.5) for 10 min at room temperature. The fixed cells were washed twice with PBS, once with the microtubule stabilizing buffer solution described by Solomon [18] [0.1 M Pipes (pH 6.9), 2 M glycerol, 5 mM $MgCl_2$, 2 mM EGTA], and permeabilized for 15 min at room temperature in the same buffer solution supplemented with 0.1% (v/v) Triton X-100. After two additional washes in PBS, the cells were incubated for 1 hr at room temperature with monoclonal murine anti-β-tubulin antibody (1:200 dilution in PBS). The cells were washed twice more with PBS and stained for 1 hr at 37° with polyclonal goat $F(ab')_2$ anti-mouse IgG conjugated to Texas red (1:200 dilution in PBS) and with 0.1% (w/v) 4,6-diamidino-2-phenylindole (DAPI), which stains DNA. The cells were washed twice more with PBS and once with water. The chambers were disassembled, and the coverslips mounted on slides with aqueous mounting medium (from Signet Laboratories). The slides were examined with a Nikon Optiphot-2-microscope equipped with epifluorescence and a rhodamine filter. Photographs were taken with a Nikon model UFX-DX camera using Kodak Tri-X film.

RESULTS

Antimitotic effects of dolastatin 15. Initial evaluation of natural dolastatin 15 demonstrated its potent toxicity for cells in culture [5]. When the depsipeptide was successfully synthesized [11], we confirmed with L1210 murine leukemia cells that it had activity comparable to the natural product (also see below). Accumulation of mitotic cells as a function of dolastatin 15 concentration in the treated cultures was closely correlated with growth inhibition with both natural and synthetic depsipeptide (the maximum proportion of mitotic cells was about 40% in these studies). Comparing the effects of dolastatin 15 with those of dolastatin 10 and vinblastine on the growth of L1210 murine leukemia cells, we obtained IC_{50} values of 3, 0.4, and 20 nM for the three drugs, respectively.* Dolastatin 15 is thus about one-seventh as active as dolastatin 10 and 7-fold more active than vinblastine as an inhibitor of this cell line.

With all three drugs at concentrations near the IC_{50} values we observed many cells which had two or more small nuclei in addition to mitotic cells with condensed chromosomes. Such cells, following treatment with dolastatin 15, are shown in Fig. 2B (cf. untreated cells, Fig. 2A). At the highest drug concentrations examined, the bi- and multinucleated cells almost disappeared, and mitotic cells were prominent. Figure 2C shows cells treated with a high concentration of dolastatin 15.

Effects of dolastatin 15 on the growth of Burkitt lymphoma cells (a human line) have also been evaluated. IC_{50} values in the 2–3 nM range have been routinely obtained, and the proportion of cells arrested in mitosis has been as high as 70%. Unlike the L1210 cells, however, mitotic cells predominated in cultures of Burkitt cells at all dolastatin 15 concentrations, and few binucleated or multinucleated cells were seen.

Interactions of dolastatin 15 *with tubulin.* In our preliminary evaluation of natural dolastatin 15, we had found that it had definite but feeble inhibitory effects on the glutamate-induced polymerization of purified tubulin. When the synthetic agent became available, it was directly compared to the natural product (Fig. 3), and the two were identical in this assay as well as in the L1210 study discussed above. Four concentration studies (two each with the natural and synthetic depsipeptides) yielded an average IC_{50} value of 23 (±1, SD) μM. This is a surprisingly high value for an antimitotic natural product. For comparison, we have obtained the following values for agents binding in the vinca domain† of tubulin: 1.2 μM for dolastatin 10, 1.4 μM for phomopsin A, 1.5 μM for vinblastine, 3.4 μM for maytansine, 6.9 μM for rhizoxin, and 7.2 μM for halichondrin B [7, 19]. Homohalichondrin B, however, does have an IC_{50} value just over 15 μM, not too much lower

* Multiple determinations of the IC_{50} value of dolastatin 10 have yielded values ranging from 0.3 to 0.9 nM [7, 9].

† Agents which inhibit the binding of vinblastine and vincristine to tubulin either competitively (maytansine, rhizoxin) or noncompetitively (dolastatin 10, phomopsin A, halichondrin B) interfere with nucleotide exchange on tubulin [8, 19]. In addition, all these drugs, as well as GTP but not vinblastine, strongly inhibit formation of an intra-β-tubulin cross-link between two cysteine residues in nucleotide-depleted tubulin [20, 21]. We have therefore proposed that the noncompetitive inhibitors, including the peptides dolastatin 10 and phomopsin A, bind in close proximity to the vinca and exchangeable GTP sites on tubulin, sterically (as opposed to allosterically) blocking access to these sites by their ligands. We have suggested that this region of the tubulin molecule be called the "vinca domain", as opposed to the more specific "vinca site" and "peptide site" where the vinca alkaloids and peptide antimitotic agents bind directly.

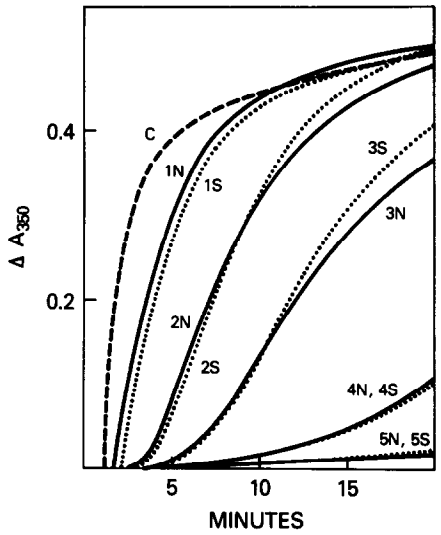


Fig. 3. Comparison of natural and synthetic dolastatin 15 on the glutamate-induced polymerization of purified tubulin. Each reaction mixture contained 1.0 mg/mL tubulin, 1.0 M monosodium glutamate (pH of 2 M stock solution adjusted to pH 6.6 with HCl), 1.0 mM $MgCl_2$, 4% (v/v) dimethyl sulfoxide, and drug as indicated below in a 0.24 mL volume (concentrations refer to the final reaction volume of 0.25 mL). Samples were incubated for 15 min at 37° and chilled on ice, and 10 μL of 10 mM GTP was added to each reaction mixture. Polymerization was followed turbidimetrically in Gilford spectrophotometers following a 75-sec temperature jump from 0° to 37° (initiation of the jump at zero time). The reaction mixture without drug is represented by the dashed curve labeled C, those containing natural dolastatin 15 by the solid curves labeled N, and those containing synthetic dolastatin 15 by the dotted curves labeled S. Dolastatin 15 concentrations were as follows: curves 1, 10 μM; curves 2, 15 μM; curves 3; 20 μM; curves 4, 25 μM; and curves 5, 30 μM.

than that of dolastatin 15 [19]. In particular, the IC_{50} value for dolastatin 15 was about 20-fold greater than that of dolastatin 10.

In preliminary experiments dolastatin 15 was also examined for its effect on tubulin polymerization dependent on microtubule-associated proteins (MAPs). There appeared to be little quantitative difference in the effect of the depsipeptide on tubulin polymerization with MAPs as compared with its effect on the glutamate-induced reaction (data not presented). This was similar to the results we obtained previously with dolastatin 10 and vinblastine [7]. In contrast, halichondrin B and homohalichondrin B appeared to have lower IC_{50} values with MAPs as opposed to glutamate [19].

The structural analogy of dolastatin 15 to dolastatin 10 indicates that the two agents may share a binding site on tubulin, despite the large difference in their effects as inhibitors of polymerization. Dolastatin 10 does not inhibit the binding of radiolabeled colchicine to tubulin, but it does noncompetitively inhibit the binding of radiolabeled vincristine and vinblastine to tubulin. Dolastatin 10 also inhibits nucleotide exchange on β-tubulin and tubulin-dependent GTP hydrolysis, and it stabilizes the colchicine binding site on tubulin [7, 8]. We thus anticipated that dolastatin 15 would bind in the peptide site of tubulin, which we postulate interacts with the vinca site to form the vinca domain [8].

With dolastatin 15, however, we were unable to demonstrate inhibition of nucleotide exchange or of radiolabeled vinblastine binding, nor any stabilization of the colchicine binding activity of tubulin with drug concentrations as high as 100 μM* (data not presented). Only GTP hydrolysis was inhibited by the depsipeptide. Of the vinca domain drugs we have examined previously, rhizoxin and halichondrin B have the weakest inhibitory effects on GTP hydrolysis [19], and dolastatin 15 was compared with dolastatin 10 and rhizoxin (Fig. 4). The drug concentrations which inhibited hydrolysis by 50% were about 2 μM for dolastatin 10 (previous values, 3 and 4 μM [7, 19]), 10 μM for rhizoxin (previous values, 12 and 16 μM [7, 19]) and 33 μM for dolastatin 15. These concentrations do not differ greatly from the drug concentrations which inhibited polymerization by 50%.

Effects of dolastatin 10 *and dolastatin* 15 *on intracellular microtubules.* The apparently feeble interaction of dolastatin 15 with tubulin is highly unusual for such a potent antimitotic agent. Almost without exception such compounds inhibit tubulin polymerization (or stimulate it, in the case of taxol) at concentrations substoichiometric to the tubulin concentration [for a review, see ref. 22]. Only homohalichondrin B seems similar to dolastatin 15 in being required at superstoichiometric concentrations to inhibit tubulin polymerization despite its toxicity for cells at nanomolar concentrations [19]. We were therefore concerned that microtubules might not be the primary cellular target for dolastatin 15.

To investigate this point further we treated wild type CHO cells with different concentrations of dolastatin 15 and examined their microtubule networks by indirect immunofluorescence. For comparison, similar studies were performed with dolastatin 10 as representative of typical antitubulin agents. Initial experiments on the effects of the two drugs on the growth of CHO cells yielded IC_{50} values of about 0.5 nM for dolastatin 10 and 5 nM for dolastatin 15. When care was taken to use concentrations of the two agents with similar effects on cell growth, their effects on the microtubules of CHO cells were indistinguishable (Fig. 5).

CHO cells in untreated cultures were characterized by single nuclei, as revealed by DAPI staining (not shown), and they had the typical fibrillar array of interphase microtubules revealed by the anti-β-

* If, as the polymerization data indicate, the interaction of dolastatin 10 with tubulin is 20-fold stronger than the interaction of dolastatin 15 with the protein, then 100 μM dolastatin 15 corresponds to only 5 μM dolastatin 10. In previous studies we have found strong inhibition of the binding of radiolabeled vinblastine to tubulin by 5 μM dolastatin 10 [7] and significant inhibition (about 35%) of nucleotide exchange by 5 μM dolastatin 10 [8]. Thus far, however, we have examined stabilization of the colchicine binding activity of tubulin only at 40–50 μM dolastatin 10 [8, 19], which would correspond to 0.8–1.0 mM dolastatin 15.

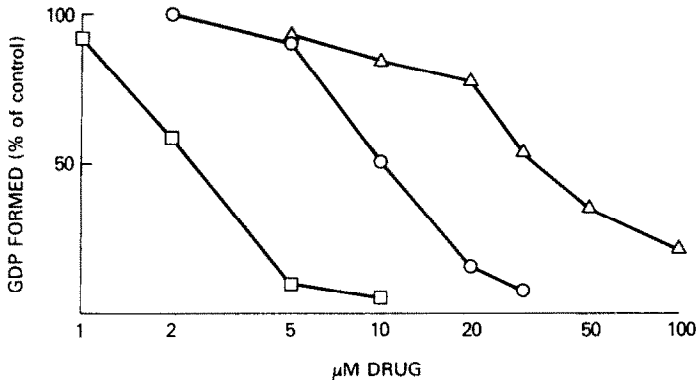


Fig. 4. Comparison of the effect of dolastatin 15 with those of dolastatin 10 and rhizoxin on tubulin-dependent GTP hydrolysis. Each 100-μL reaction mixture contained 1.0 mg/mL tubulin, 1.0 M monosodium glutamate (2 M stock solution adjusted to pH 6.6 with HCl), 100 μM [8-^{14}C]GTP, 5% (v/v) dimethyl sulfoxide, and drugs as indicated. Symbols: (△), dolastatin 15; (□) dolastatin 10; and (○) rhizoxin. Incubation was for 5 min at 37°. In the control reaction mixture (without drug) 8.2 nmol of [8-^{14}C]GDP was formed per mL of reaction mixture.

tubulin antibody (Fig. 5A). In contrast, cells treated with either dolastatin 10 or dolastatin 15 at concentrations near the IC_{50} values showed a partial disappearance of the microtubular network and were frequently multinucleated (Fig. 5, B and C). This latter finding was reminiscent of the results with L1210 cells described above. Multinucleation was clear from the DAPI fluorescence (not presented), but it is also apparent in the photomicrographs presented in Fig. 5, B and C. We should note that few mitotic cells were visualized in our preparations, either at concentrations near the IC_{50} value or at higher concentrations, and we believe they were lost during our washing procedures.

When drug concentrations 10-fold higher than the IC_{50} values were used, most of the cells had completely lost their microtubule networks (Fig. 5, D and E). This structural change was associated with disappearance of the multinucleation phenomenon. Cells without microtubules almost always had a single nucleus when visualized under DAPI (not presented). This observation can be compared to those made of the L1210 cultures, where the maximum mitotic index was about 40%, and few multinucleated cells were observed at the highest drug concentrations examined.

DISCUSSION

In this report we have described our initial findings with dolastatin 15, a depsipeptide originally isolated from *D. auricularia* [5]. Dolastatin 15 appears to derive its toxicity toward cells in culture by interfering with mitosis. Presumably this results from a specific interaction of the drug with tubulin, resulting in inhibition of microtubule assembly. Dolastatin 15, however, is unusual for such a toxic antimitotic agent in that its *in vitro* interactions with tubulin appear to be feeble. The most convincing evidence that dolastatin 15 is an inhibitor of tubulin polymerization was the disappearance of cellular microtubules in cells treated with relatively high concentrations of the drug (Fig. 5).

Dolastatin 15 shares many structural features with dolastatin 10, but it is less cytotoxic than the latter, and at this point it appears to be 20-fold weaker in its interaction with tubulin (based on relative inhibitory effects on tubulin polymerization in glutamate). Its structural similarity to dolastatin 10 and its feeble inhibitory effect on tubulin-dependent GTP hydrolysis indicate that dolastatin 15, too, binds in the vinca domain of tubulin, presumably in the same site as dolastatin 10 (termed the peptide site, see Ref. 8). Since we cannot demonstrate any inhibition by dolastatin 15 of the binding of radiolabeled vinblastine to tubulin, this is only a preliminary conclusion. The failure of dolastatin 15 to affect the binding of radiolabeled GTP or vinblastine to tubulin may reflect its weak effect on polymerization.

We are attempting to gain insights into critical features of the structures of dolastatins 10 and 15 required for effective interaction of these compounds with tubulin and for effective cytotoxic activity. Our approach includes the synthesis and examination of chiral isomers, of modified peptides, and of segments of these compounds. Most of this work has been focused on dolastatin 10 (but see below). Our initial studies [9] demonstrated that the native configuration at C(18) and C(19), but not at C(9) and C(10), is required for inhibition of tubulin polymerization. Maximum cytotoxicity, however, requires the native configuration at all four positions [9]. Findings with newer dolastatin 10 analogs further support these conclusions, and they have also demonstrated that the dolaphenine residue is relatively unimportant for inhibition of either tubulin polymerization or cell growth (manuscript in preparation). Besides the segment consisting of residues 1–3 of dolastatin 10 which had activity as an inhibitor of polymerization

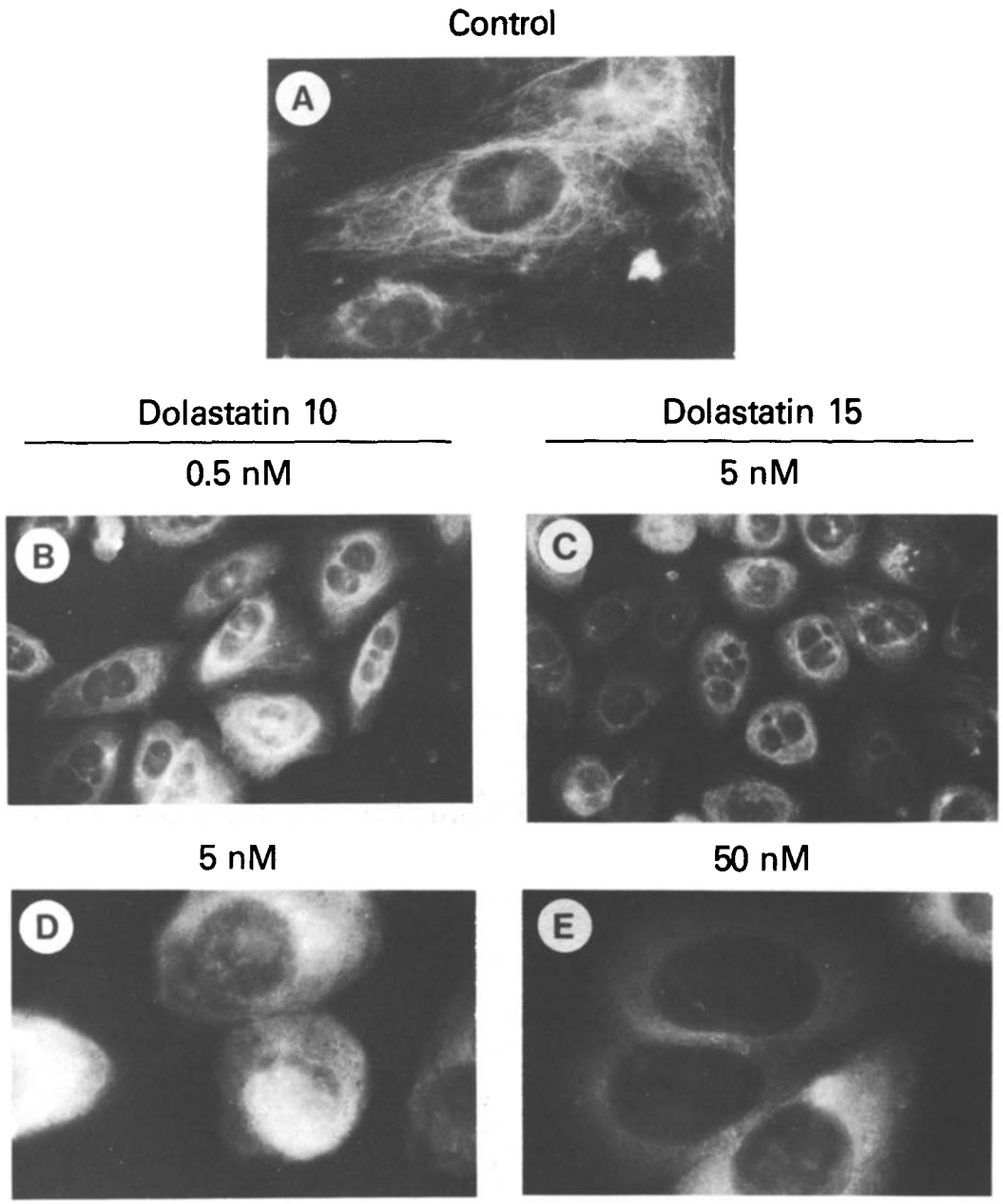


Fig. 5. Comparison of the effects of dolastatin 15 with those of dolastatin 10 on the microtubule network of CHO cells at comparably toxic concentrations. Cells were grown on eight chamber glass slides for 24 hr at 37° and subsequently treated with drugs as indicated for an additional 16 hr. After fixation, microtubules were visualized by sequential treatment with a murine monoclonal antibody to β-tubulin and a Texas red labeled goat polyclonal $F(ab')_2$ antibody to murine IgG. (A) Untreated CHO cells. (B) CHO cells treated with the IC_{50} concentration (0.5 nM) of dolastatin 10. (C) CHO cells treated with the IC_{50} concentration (5 nM) of dolastatin 15. (D) CHO cells treated with ten times the IC_{50} concentration (5 nM) of dolastatin 10. (E) CHO cells treated with ten times the IC_{50} concentration (50 nM) of dolastatin 15. For the photographs presented in panels A, D, and E, magnification was ×1200. For the photographs presented in panels B and C, magnification was ×470.

[9], inhibitory activity has now also been obtained in segments containing residues 3–5 and, especially, residues 2–5, but no segment was strongly cytotoxic (manuscript in preparation). Thus, the dolavaline and dolaproine residues appear to be less important for an interaction with tubulin but critical for cytotoxicity. The reason(s) for this divergence is presently unknown.

Table 1. Effects of vinca domain drugs on cell proliferation and tubulin polymerization*

Drug	Inhibition of cell proliferation IC_{50} (nM)	Inhibition of tubulin polymerization IC_{50} (μM)
Dolastatin 15	3	23
Dolastatin 10	0.5	1.2
Isomer 1	90	1.3
Isomer 3	600	1.2
Isomer 8	40	28
Isomer 15	600	>40
Halichondrin B	0.3	7.2
Homohalichondrin B	1	15–20
Phomopsin A	7000	1.4
Vinblastine	20	1.5
Maytansine	0.5	3.4
Rhizoxin	1	6.9

* The data for dolastatin 15 are from studies here. For the other agents, data are summarized from Refs. 2, 8, 9, and 19. In a few cases averages of similar values obtained in different studies are presented in the table, including those obtained in studies summarized in this paper. In isomers of dolastatin 10, configuration at asymmetric carbon atoms was reversed as follows: for Isomer 1, at position 10; for Isomer 3, at positions 9 and 19a; for Isomer 8, at positions 18, 19, and 19a; and for Isomer 15, at positions 9, 18, 19, and 19a [9].

If dolastatin 15 does bind in the same site on tubulin as dolastatin 10, these observations, together with initial negative findings with large segments of dolastatin 15 (see below), suggest that at least the first six residues of the depsipeptide will be required for activity. Dolapyrrolidone presumably would be as unimportant as dolaphenine. Perhaps the first proline residue of dolastatin 15 corresponds to positions C(17) and C(18) of dolastatin 10, while the 2-hydroxyisovalerate residue corresponds to positions C(9) and C(10).

Although dolastatin 15 at this point seems to represent an extreme for antimitotic agents in terms of its weak effect on tubulin polymerization, there has been relatively limited correlation between inhibition of cell proliferation (L1210 murine leukemia cells) and inhibition of tubulin polymerization (glutamate-induced) among the vinca domain drugs we have examined (Table 1). If one considers homohalichondrin B [19] and, especially, chiral isomers of dolastatin 10 [9], then similar disjunctions have been observed with other agents.

There are several possible explanations for this generally limited correlation of antitubulin and antiproliferative effects, and for the specific disjunction observed with dolastatin 15. For example, agents such as dolastatin 15 with relatively weak interactions with tubulin could be transported into cells with high efficiency, resulting in sufficient intracellular concentrations to disrupt microtubule assembly despite their relatively deficient interaction with tubulin. Alternatively, the mechanism of intracellular microtubule assembly may differ significantly from that which occurs *in vitro*.

Finally, we should note the possibility of intracellular metabolism of dolastatin 15 to a more active compound. For example, its ester bond could be readily hydrolyzed and either the hydrolysis product(s) or their metabolites could be potent inhibitors of microtubule assembly. Pertinent to this possibility is the *in vitro* antitubulin activity of segments of dolastatin 10 ([8, 9], manuscript in preparation). Thus far, however, none of the synthetic segments of dolastatin 15 that we have examined affects *in vitro* tubulin polymerization at 100 μM. The four largest of these inactive segments contained residues 1–4, residues 2–5, residues 5–7, and residues 2–7. Simple intracellular fragmentation of dolastatin 15 is thus unlikely to explain its potent antimitotic activity, but we cannot at present exclude other metabolic modifications of the depsipeptide.

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