(-)-Doliculide, a New Macroyclic Depsipeptide Enhancer of Actin Assembly*

Ruoli Bai‡, David G. Covell‡, Chunfeng Liu‡, Arun K. Ghosh‡, and Ernest Hamel¶

From the Screening Technologies Branch, Developmental Therapeutics Program, Division of Cancer Treatment and Diagnosis, NCI National Institutes of Health, Frederick, Maryland 21702 and the Department of Chemistry, University of Illinois, Chicago, Illinois 60607

The cytotoxic, cyclic depsipeptide (-)-doliculide was originally isolated by Ishiwata et al. (Ishiwata, H., Nemoto, T., Ojika, M., and Yamada, K. (1994) J. Org. Chem. 59, 4710–4711 and Ishiwata, H., Sone, H., Kigoshi, H., and Yamada, K. (1994) J. Org. Chem. 59, 4712–4713) from the sea hare Dolabella auricularia collected in Japanese waters, but the mechanism of action of the depsipeptide was not known. Using synthetic (-)-doliculide, we found that the compound arrests cells at the G2/M phase of the cell cycle by interfering with normal actin assembly. In cells, normal stress fibers disappeared and were replaced by multiple clumps of apparently aggregated F-actin. These effects of (-)-doliculide on cells were essentially identical to those obtained with jasplakinolide. Like jasplakinolide, (-)-doliculide caused the hyperassembly of purified actin into F-actin as measured both fluorometrically and by centrifugation. In addition, (-)-doliculide, like jasplakinolide, readily displaced a fluorescent phallolidin derivative from actin polymer. In these biochemical assays (-)-doliculide and jasplakinolide were quantitatively virtually identical in their behaviors. Similar effects have also been reported with a series of depsipeptides known as chondramides. Using recently developed, computer-driven shape descriptor analysis (Mansfield, M. L., Covell, D. G., and Jernigan, R. L. (2002) J. Chem. Inf. Comput. Sci. 42, 259–273), we compared (-)-doliculide with jasplakinolide, phallolidin, and chondramide C to gain insight into a possible pharmacophore that would explain the apparent binding of this diverse group of molecules at the same site on F-actin. We found that the segment of (-)-doliculide that best overlapped the other molecules encompassed its phenyl and isopropyl side chains and the portion of the macrocycle between these substituents.

The sea hare Dolabella auricularia has been a rich source of cytotoxic peptides and depsipeptides (see Refs. 1 and 2 for reviews). Interestingly, structurally different molecules have been derived from Indian Ocean (for example, dolastatins 10 (3), 11 (4), and 15 (5)) and Pacific Ocean (for example, dolastatin G (6) and dolastatin H (7)) specimens of the organism, and it seems increasingly likely that these peptides and depsipeptides may actually derive from cyanobacteria that D. auricularia shelters or upon which it feeds (8, 9). Although Yamada and co-workers (10–12) described the isolation, cytotoxic activity, and synthesis of (-)-doliculide1 (see structure in Fig. 1) in 1994, its mechanism of action has remained unknown. A new synthesis of doliculide (13) allowed us to investigate its effects on cultured cells and to explore the possibility that the compound interfered with the assembly of purified tubulin or actin, the cellular targets of other Dolabella auricularia-derived peptides (14–16).

We found that doliculide, like jasplakinolide (17, 18), the chondramides (19), and phallolidin (structures in Fig. 1; stereochemistry for phallolidin as described in Ref. 20) potently enhanced the assembly of purified actin and inhibited the binding of FITC2-labeled phallolidin to actin polymer. Treatment of cells with doliculide caused them to arrest at cytokinesis and caused substantial rearrangement of intracellular F-actin. The structural similarities and differences between doliculide and other compounds that seem to bind at the same site on F-actin led us to search for a common pharmacophore by molecular modeling techniques.

EXPERIMENTAL PROCEDURES

Materials—Doliculide was prepared as described previously (13). Actin and pyrenyl-labeled actin from rabbit muscle were obtained from Cytokeleton, Inc.; jasplakinolide from the Drug Synthesis and Chemistry Branch, NCI National Institutes of Health; phallolidin and anti-fade mounting solution from Molecular Probes; human Burkitt lymphoma CA46 cells and PtK2 cells (normal kidney epithelial cells of the kangaroo rat Potorous tridactylus) from the American Type Tissue Collection; DAPI, FITC-conjugated phallolidin, and FITC-conjugated anti-β-actin monoclonal antibody from Sigma; and the Lab-Tek II chamber slide with cover from Nalge Nunc International. Dolastatin 11 was generously provided by Dr. R. B. Bates, University of Arizona.

Methods—CA46 and PtK2 cells were maintained in culture as recommended by the supplier. Drug effects on growth were evaluated by an increase in cell protein as described previously (21). Cells were grown at 37 °C in a humidified 5% CO2 atmosphere. For flow cytometry and immunofluorescence studies, the cells were grown to confluence, disrupted by trypsinization, and seeded at about 10% confluence. For flow cytometric studies the cells were grown in 50-ml tissue culture flasks, whereas for immunofluorescence studies they were grown in the Lab-Tek II chamber slide.

For flow cytometry CA46 cells were treated for 72 h with doliculide at the IC50 (30 nM) or with a 10-fold higher concentration in 1% dimethyl sulfoxide or with the solvent only as a control. The cells were harvested by centrifugation, resuspended in PBS, and fixed in 70% ethanol for 30 min at 4 °C. The cells were recollected by centrifugation and suspended in 1 ml of PBS containing 100 mg each of propidium iodide and RNase A. DNA content of the cells was analyzed with a Becton Dickinson FACScan flow cytometer, and the proportion of cells in G2/M was quantitated by peak integration using Modfit LT V2.0 software.

* The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked "advertisement" in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

¶ To whom correspondence should be addressed: NCI National Institutes of Health, Bldg. 469, Rm. 104, Frederick, MD 21702. Tel.: 301-846-1678; Fax: 301-846-6014; E-mail: hamele@mail.nih.gov.

1 Hereinafter referred to simply as “doliculide.”

2 The abbreviations used are: FITC, fluorescein isothiocyanate; DAPI, 4’,6-diamidino-2-phenylindole; PBS, phosphate-buffered saline.
For immunofluorescence studies the cells were grown for 2–3 days prior to drug treatment (final dimethyl sulfoxide concentration, 1% (v/v)), and cells were left at 37 °C for the indicated additional time periods. Cells were washed twice with PBS, fixed with methanol at −20 °C for 10 min (FITC-actin antibody) or with 3.7% formaldehyde in PBS for 5 min at 22 °C (FITC-phalloidin), washed twice with PBS, treated with −20 °C acetone for 1 min, treated with 0.1% Triton X-100 at 22 °C (FITC-phalloidin only), and washed twice with PBS. DAPI at 1.0 μg/ml and either the FITC-conjugated anti-β-actin antibody (diluted 1:250 with PBS) or the FITC-conjugated phalloidin at 50 μg/ml were added in 150 μl of PBS to the cells on the chamber slide, which was left for 1 h at 22 °C in the dark. The chamber slide was washed twice with PBS, the specimen was air-dried, and a coverslip was applied with antifade mounting solution. The cells were examined with a Nikon Model Eclipse E800 microscope equipped with epifluorescence and appropriate filters. Images were captured with a Spot digital camera, model 2.3.0, using version 3.0.2 software (Diagnostic Instruments). All images shown here were obtained with a 40× oil objective (N.A. 1.30).

For fluorometric evaluation of actin assembly, a Photon Technology International fluorometer was used with Felix for Windows software. Actin and pyrenyl-labeled actin were diluted as a mixture to 12.5 and 1.0 μM, respectively, in a solution containing 5 mM Tris-HCl (pH 8.0), 0.2 mM CaCl2, 0.2 mM ATP, and 5 mM dithiothreitol. After 1 h at 0 °C the mixture was centrifuged at 45,000 rpm in a Beckman Ti70 rotor at 4 °C. The mixture was then diluted to 10 μM total actin in the same solution in a 100-μl volume and transferred to a fluorescence cuvette at 22 °C. The fluorescence signal was referenced, drug in 1 μl of dimethyl sulfoxide was added, and fluorescence was monitored (excitation at 365 nm, emission at 407 nm).

For centrifugal evaluation of actin assembly (see Ref. 16 for further details), actin was diluted to 25 μM in the above solution and left at 0 °C for 1 h. Various concentrations of drug in 5 μl of dimethyl sulfoxide were added to 95-μl aliquots of the actin solution. Reaction mixtures were incubated for 2 h at 22 °C and centrifuged for 30 min at 45,000 rpm in a Beckman TLA 55 rotor in an Optima TLX micro-ultracentrifuge at 22 °C. The protein content of the supernatants was determined by the Lowry method with the EC50 of a drug defined as the concentration that reduces the supernatant actin concentration by 50% relative to controls without drug.

The inhibition of binding of FITC-phalloidin to actin polymer was measured by removing polymer from the 100-μl reaction mixtures by centrifugation followed by measurement of fluorescence of the supernatant.
Results

Cellular Studies—After initial screening experiments in which doliculide was examined for effects on tubulin (inactive; data not presented) and actin (active; see below) assembly, the effects of the depsipeptide on the growth of CA46 and PtK2 cells were determined. Nearly identical IC50 values were obtained: 30 nM for the former cell line and 40 nM for the latter. In contemporaneous studies with jasplakinolide in the PtK2 cells an IC50 value of 40 nM was also obtained.

The Burkitt cells were examined for cell cycle effects of doliculide (Fig. 2). At the IC50 value (30 nM) a slight increase in the fraction of cells arrested in both the G2/M and S phases of the cell cycle was observed. At a 10-fold higher concentration, however, there was a large increase in cells arrested at G2/M, and a significant number of octoploid cells were also observed.

The PtK2 cells were examined by immunofluorescence for cytoskeletal changes caused by doliculide (Figs. 3 and 4). In these studies doliculide was compared with jasplakinolide. We found only minor effects, presumably secondary, for treatment with either drug on the appearance of microtubules in these cells (data not presented). The actin cytoskeleton was examined both with FITC-conjugated phalloidin and with an FITC-conjugated anti-β-actin monoclonal antibody. Because the appearance of the cells was indistinguishable with both fluorescent agents, only results obtained with the antibody are presented here.

Fig. 3 shows untreated control cells (A) and cells treated with doliculide (B and C) or jasplakinolide (D and E) for 16 h at either 40 nM (the IC50 value for both drugs, B and D) or at 400 nM (C and E). At this time point the effects of the two drugs were indistinguishable although there were dramatic differences observed at the two concentrations examined. At 40 nM there was partial clumping of F-actin, but the stress fiber architecture of the cells seemed relatively unaffected by treatment with either drug. At the higher drug concentration, the stress fibers had completely disassembled from all cells, and there was extensive clumping of the F-actin. There was also extensive cytoplasmic retraction, more obvious with the tubulin-stained cells than in those stained for actin shown here.

Binucleate cells, indicated by arrows, were seen frequently after 16 h of treatment with either drug. However, there was little difference, relative to the control, in the number of mitotic cells (those with condensed chromosomes, as indicated by the DAPI fluorescence).

A more detailed time course analysis was performed with the drugs at 400 nM (Fig. 4). With doliculide the stress fibers had almost completely disappeared with only 30 min of drug treatment (A). By 2 h (B) the F-actin clumping was almost as extensive as after 16 h although cytoplasmic retraction was not yet extensive. With jasplakinolide there was greater retention of stress fibers at the 30 min time point (C), and even after 2 h (D) scattered stress fibers persisted. As with doliculide cytoplasmic retraction was not yet dramatic at 2 h. With both drugs only rare binucleate cells were seen at these early time points.

Studies with Purified Actin—The similarity of the effects of doliculide and jasplakinolide on the cultured cells was also observed in the effects of the two compounds on actin. Polymerization was examined both by the standard fluorescence assay (copolymersation of actin and pyrenyl-actin) (23) and by a centrifugal assay described previously (16).

In the fluorescence assay we examined a low ionic strength reaction condition in which assembly is minimal in the absence of drug (Fig. 5). The extent of fluorescence induced by doliculide was greater than that obtained with jasplakinolide at most concentrations examined. There is an apparent concentration dependence in extent of fluorescence observed with both drugs. Although the greater fluorescence observed with doliculide...
could indicate more extensive assembly induced with this agent as compared with jasplakinolide, it is also possible that the two drugs could cause differences in fluorescence intensity of the polymerized pyrenyl-actin.

This latter possibility is consistent with similar amounts of polymer harvested with both drugs as a function of drug concentration. In the centrifugal assay, also performed at low ionic strength, almost identical EC$_{50}$ values were obtained (20±3 μM for doliculide; 23±1 μM for jasplakinolide). In this assay we define the EC$_{50}$ value as the concentration of drug required to reduce the supernatant actin concentration (relative to control reaction mixtures without drug) by 50%, following centrifugation for 30 min at 45,000 rpm.

We next examined the ability of doliculide to inhibit the binding of FITC-labeled phalloidin to actin polymer by measuring fluorescence of the supernatant following centrifugation. In these experiments, performed at higher ionic strength (50 mM KCl plus 2 mM MgCl$_2$), the effect of doliculide was com-

---

**Fig. 3.** Comparison of the effects of doliculide with those of jasplakinolide on the actin cytoskeleton of PtK2 cells. Cells were grown for 16 h with drug, as indicated, and the actin cytoskeleton was visualized as described under “Experimental Procedures.” A, no drug. B, doliculide at 40 nM (the IC$_{50}$ value). C, doliculide at 400 nM. D, jasplakinolide at 40 nM (the IC$_{50}$ value). E, jasplakinolide at 400 nM.

**Fig. 4.** Comparison of the effects of different treatment times with doliculide or jasplakinolide on the actin cytoskeleton of PtK2 cells. Cells were treated for the indicated times with the indicated drug at 400 nM, and the actin cytoskeleton was visualized as described under “Experimental Procedures.” A, doliculide for 30 min. B, doliculide for 2 h. C, jasplakinolide for 30 min. D, jasplakinolide for 2 h.
pared with those of jasplakinolide and dolastatin 11 (Fig. 6). As previously shown, dolastatin 11 (16) was unable to prevent the binding of FITC-phalloidin to actin polymer, whereas jasplakinolide (16, 17) displaced the phalloidin analog. Quantitatively, dolastatin 11 was almost identical to jasplakinolide as an inhibitor of FITC-phalloidin binding to actin polymer. In fact, combining the results shown in Fig. 6 with those obtained in previous studies (16, 17, 19), there appears to be little quantitative difference in the potency of all compounds (phalloidin, jasplakinolide, chondramide A, doliculide) thus far shown to inhibit the interaction of fluorescent phalloidin derivatives with actin polymer.

Pharmacophore Modeling—The quantitatively similar effects of phalloidin, jasplakinolide, chondramide A, and doliculide in their interactions with actin polymer have encouraged us to try to find a common pharmacophore in these four compounds. Besides their oligopeptidic structure, there is no obvious common feature except that all four molecules contain macrocycles, which vary in size. The macrocycle of jasplakinolide contains 19 atoms, that of chondramide A 18 atoms, that of dolastatin 11 16 atoms, and the two fused macrocycles of phallolidin have 15 and 18 atoms. Phallolidin differs from the other three compounds in lacking a phenyl ring, multiple closely spaced methyl groups attached to the macrocycle, and an ester bond in the macrocycle. Doliculide differs from the other structures in lacking an indole ring. It would therefore appear that these structural features are not essential for the interaction of this group of compounds with polymerized actin in the phallolidin binding site.

Using our recently developed algorithm (22) that allows computational comparison of molecules for similar three-dimensional shapes, an analysis was performed to determine the consensus set of atoms within a subvolume of the shape-matched alignment. Because the stereochemistry at the chiral centers of the chondramides has not been determined (19, 24), we substituted chondramide C for chondramide A for this analysis. It has one less chiral center and appears to have close structural analogy to jasplakinolide. We arbitrarily assigned configurations to the chiral centers of chondramide C that seemed equivalent to those of apparently homologous centers in jasplakinolide (Fig. 1).

The computational analysis yields numerical comparisons between molecules taken two at a time termed goodness-of-fit-similar-shape values. All pairs between dolastatin, phallolidin, jasplakinolide, chondramide C, and dolastatin 11 were analyzed, and the results are shown in Table I. Negative values indicate significant shape overlap, with more negative values generally meaning more overlap (however, see “Experimental Procedures”), whereas positive values indicate essentially random matching.

The best shape overlap was, not surprisingly, between chondramide C and jasplakinolide, with the dolastane-chondramide C pair the next best match. In descending order the remaining pairs were phallolidin-jasplakinolide, phallolidin-chondramide C, doliculide-jasplakinolide, and doliculide-phallolidin. Dolastatin 11 had poor shape overlap scores, i.e. positive goodness-of-fit-similar-shape values, with all of the other molecules.

Fig. 7 shows the shape overlap pairs of dolastatin with jasplakinolide, phallolidin, or chondramide C. The molecules of each pair are separated in space but shown in their overlap orientations. In addition, we show as spheres atoms of dolastane matched with atoms of each of the other two compounds within the sum of their van der Waals radii. Unexpectedly, virtually every atom of dolastane matched an atom in at least one of the other three peptides, with the benzyl group, the isopropyl group, and the portion of the macrocycle linking these substituents largely matching atoms in all three peptides. This segment of dolastane thus would be a good candidate for the core of the pharmacophore. From this analysis, the phenyl ring of dolastane largely corresponds to the indole ring of jasplakinolide, phallolidin, and chondramide C. The isopropyl group of dolastane corresponds to the phenyl ring of jasplakinolide, to a portion of the macrocycle backbone adjacent to the prolyl residue of phallolidin, and to a portion of the macrocycle adjacent to the ester bond of chondramide C. It is also worth noting that, from this analysis, the iodine atom of dolastane overlaps reasonably well with the bromine atom of jasplakinolide (6.6 Å separates their atomic centers in the structures shown in Fig. 7).

DISCUSSION

Doliculide, like dolastatin 11 (4), is a depsipeptide originally extracted from the sea hare D. auricularia (10), but the source organism was obtained from Pacific Ocean waters rather than from the Indian Ocean. We have shown here that dolastane, like dolastatin 11 (16), jasplakinolide (17), phallolidin (23, 25), and chondramide A (19), enhances the assembly of purified...
actin, arrests cells at cytokinesis, and causes the accumulation of presumptive (based on the binding of FITC-phalloidin) F-actin aggregates in cells treated with the drug. This F-actin clumping phenomenon has been speculated to represent bundling of very short actin filaments formed as a result of hypernucleation of actin assembly caused by these drugs (18). Dolichulide, as well as phalloidin, jasplakinolide, and chondramide A, differ from dolastatin 11 in that the latter compound has no ability to displace FITC-phalloidin from actin polymer.

Yamada and colleagues (10) noted the structural similarity of dolichulide to jasplakinolide prior to the original descriptions (17, 26) of the latter compound’s actin-based mechanism of action. In the studies presented here, we have shown that quantitatively dolichulide and jasplakinolide are almost indistinguishable in their interactions with purified actin and in their effects on the growth of PtK2 cells. This is in contrast to dolastatin 11, which was unable to displace FITC-phalloidin from actin polymer even though it was otherwise somewhat more potent than jasplakinolide in its effects on cells and actin assembly (16).

Besides the quantitative similarity between the interactions of dolichulide and jasplakinolide with actin reported here, we previously observed nearly identical ability in jasplakinolide and phalloidin in displacing FITC-phalloidin from actin polymer (16). Similarly, Sasse et al. (19) found that chondramide A and phalloidin differed little in their ability to displace a fluorescent phalloidin derivative from actin polymer. These observations suggest nearly identical affinity of the four compounds (doliculide, jasplakinolide, chondramide A, phalloidin) for actin polymer and encouraged us to search for a common pharmacophore among these peptides.

The negative goodness-of-shape-similar-fit values for all pairs of dolichulide, phalloidin, jasplakinolide, and chondramide C, and the positive values obtained from the pairing of these four peptides with dolastatin 11 are consistent with the biochemical data. We would therefore suggest that dolichulide, as well as jasplakinolide and the chondramides, would bind in the phalloidin site on F-actin (27), whereas dolastatin 11 would bind elsewhere. In fact, recent x-ray fiber diffraction analysis of F-actin containing bound dolastatin 11 has demonstrated a distinct binding site on polymer for this depsipeptide as compared with phalloidin.3

From the modeling studies, almost every atom of dolichulide overlaps with atoms in either jasplakinolide, phalloidin, or chondramide C. Atoms from the benzyl and isopropyl substituents and from the macrocycle between these substituents, however, overlap with atoms in all the other three peptides and so might be considered a “core” pharmacophore. Of particular note is that the benzyl group of dolichulide corresponds to the indole group of the other peptides and that the iodine atom of dolichulide overlaps reasonably well with the bromine atom of jasplakinolide (6.6 Å separates their atomic centers). This would be consistent with the observation of Ishiwata et al. (12) that the iodine atom is essential for the cytotoxic properties of dolichulide. Also in agreement with the structure-activity studies of Ishiwata et al. (12), our modeling study predicts that synthetic efforts to improve on the biological activity of dolichulide will not be rewarding.

3 T. Oda and K. C. Holmes, personal communication.

### Table I

|              | Dolichulide | Phalloidin | Jasplakinolide | Chondramide C |
|--------------|-------------|------------|----------------|---------------|
| Dolichulide  | −0.44       | −0.66      | −0.88          |               |
| Phalloidin   | −0.73       | −0.61      | −0.95          | −0.61         |
| Jasplakinolide| +1.77       | +1.04      | +1.15          | +1.58         |
| Dolastatin 11| +1.04       | +1.15      | +1.58          |               |
REFERENCES

1. Pettit, G. R. (1997) *Prog. Chem. Org. Nat. Prod.* **70**, 1–79
2. Poncelet, J. (1999) *Curr. Pharm. Des.* **5**, 139–162
3. Pettit, G. R., Kamano, Y., Herald, C. L., Tuinman, A. A., Boettner, F. E., Kiru, H., Schmidt, J. M., Barzynsky, L., Tomer, K. B., and Bontems, R. J. (1987) *J. Am. Chem. Soc.* **109**, 6883–6885
4. Pettit, G. R., Kamano, Y., Kiru, H., Dufresne, C., Herald, C. L., Bontems, R. J., Schmidt, J. M., Boettner, F. E., and Nieman, R. A. (1989) *Heterocycles* **28**, 553–558
5. Pettit, G. R., Kamano, Y., Dufresne, C., Cerny, R. L., Herald, C. L., and Schmidt, J. M. (1989) *J. Org. Chem.* **54**, 6005–6006
6. Mutou, T., Kondo, T., Ojika, M., and Yamada, K. (1996) *J. Org. Chem.* **61**, 6340–6345
7. Sone, H., Shiibata, T., Fujita, T., Ojika, M., and Yamada, K. (1996) *J. Am. Chem. Soc.* **118**, 1874–1880
8. Harrigan, G. G., Moore, R. E., Nagle, D. G., Park, P. U., Biggs, J., Paul, V. J., Mooberry, S. L., Corbett, T. H., and Valeriote, F. A. (1998) *J. Nat. Prod.* **61**, 1221–1225
9. Harrigan, G. G., Luess, H., Yoshida, W. Y., Moore, R. E., Nagle, D. G., Paul, V. J., Mooberry, S. L., Corbett, T. H., and Valeriote, F. A. (1998) *J. Nat. Prod.* **61**, 1075–1077
10. Ishiwata, H., Nemoto, T., Ojika, M., and Yamada, K. (1994) *J. Org. Chem.* **59**, 4710–4711
11. Ishiwata, H., Sone, H., Kigoshi, H., and Yamada, K. (1994) *J. Org. Chem.* **59**, 4712–4713
12. Ishiwata, H., Sone, H., Kigoshi, H., and Yamada, K. (1994) *Tetrahedron* **50**, 12853–12882
13. Ghosh, A. K., and Liu, C. (2001) *Org. Lett.* **3**, 635–638
14. Bai, R., Pettit, G. R., and Hamel, E. (1990) *J. Biol. Chem.* **265**, 17141–17149
15. Bai, R., Friedman, S. J., Pettit, G. R., and Hamel, E. (1992) *Biochem. Pharmacol.* **43**, 2637–2645
16. Bai, R., Verdier-Pinard, P., Gangwar, S., Stessman, C. C., McClure, K. J., Saussville, E. A., Pettit, G. R., Bates, R. B., and Hamel, E. (2001) *Mol. Pharmacol.* **59**, 462–469
17. Bubb, M. R., Senderowicz, A. M. J., Saussville, E. A., Duncan, K. L. K., and Korn, E. D. (1994) *J. Biol. Chem.* **269**, 14889–14871
18. Bubb, M. R., Spector, I., Beyer, B. B., and Fosen, R. M. (2000) *J. Biol. Chem.* **275**, 5163–5170
19. Sasse, F., Kunze, B., Gronewald, T. M. A., and Reichenbach, H. (1998) *J. Natl. Cancer Inst.* **90**, 1559–1563
20. Kessler, H., and Wein, T. (1991) *Liebigs Ann. Chem.* 179–184
21. Bai, R., Cichacz, Z. A., Herald, C. L., Pettit, G. R., and Hamel, E. (1993) *Mol. Pharmacol.* **44**, 757–766
22. Mansfield, M. L., Covell, D. G., and Jernigan, R. L. (2002) *J. Chem. Inf. Comput. Sci.* **42**, 259–273
23. Lee, E., Shelden, E. A., and Knecht, D. A. (1998) *Cell Motil. Cytoskeleton* **39**, 122–133
24. Kunze, B., Jansen, R., Sasse, F., Hølle, G., and Reichenbach, H. (1995) *J. Antibiotics* **48**, 1262–1266
25. Estes, J. E., Selden, L. A., and Gershman, L. C. (1981) *Biochemistry* **20**, 708–712
26. Senderowicz, A. M. J., Kaur, G., Sainz, E., Laing, C., Inman, W. D., Rodriguez, J., Crews, P., Malpeis, L., Grever, M. R., Saussville, E. A., and Duncan, K. L. K. (1995) *J. Natl. Cancer Inst.* **87**, 46–51
27. Lorenz, M., Popp, D., and Holmes, K. C. (1993) *J. Mol. Biol.* **234**, 826–836